J. Med. Chem. **1998,** *41,* 742−751

Tyrosine Kinase Inhibitors. 14. Structure−Activity Relationships for Methylamino-Substituted Derivatives of 4-[(3-Bromophenyl)amino]-6-(methylamino)-pyrido[3,4-*d*]pyrimidine (PD 158780), a Potent and Specific Inhibitor of the Tyrosine Kinase Activity of Receptors for the EGF Family of Growth Factors

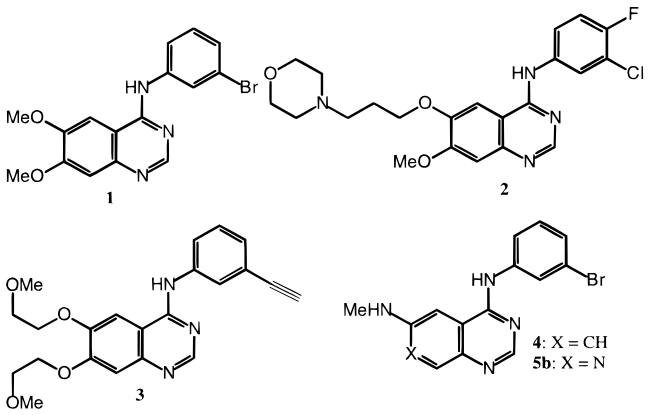
Gordon W. Rewcastle,† Donna K. Murray,† William L. Elliott,‡ David W. Fry,‡ Curtis T. Howard,‡ James M. Nelson,‡ Billy J. Roberts,‡ Patrick W. Vincent,‡ H. D. Hollis Showalter,‡ R. Thomas Winters,‡ and William A. Denny*,†

Cancer Society Research Laboratory, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92019, Auckland, New Zealand, and Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48106-1047

Received September 26, 1997

The 4-[(3-bromophenyl)amino]pyrido[3,4-*d*]pyrimidine PD 158780 is a very potent in vitro inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) (IC_{50} 0.08 nM), and other members of the *erbB* family, by competitive binding at the ATP site of these signal transduction enzymes. A series of analogues of PD 158780 bearing solubilizing functions off the 6-methylamino substituent were prepared by reaction of the 6-fluoro derivatives with appropriate amine nucleophiles. These were evaluated for their ability to inhibit the tyrosine phosphorylating action of EGF-stimulated full-length EGFR enzyme and for inhibition of autophosphorylation of the EGFR in A431 human epidermoid carcinoma cells in culture. The most effective analogues were those bearing weakly basic substituents through a secondary amine linkage, which proved water-soluble (>10 mM) and potent (IC_{50}s generally <1 nM). No clear SAR could be discerned for these compounds with respect to amine base strength or the distance of the cationic center from the chromophore, suggesting that 6-substituents are in a favorable area of bulk tolerance in the enzyme binding site. More distinct SAR emerged for the ability of the compounds to inhibit EGFR autophosphorylation in A431 cells, where analogues bearing lipophilic weak bases were preferred. Representative analogues were evaluated for antitumor effectiveness against four in vivo tumor models. Significant in vivo activity was observed in estrogen-dependent MCF-7 breast and A431 epidermoid tumors. Marginal activity was seen in an EGFR-transfected tumor model, suggesting that while this cell line requires EGF for clone formation in soft agar, other growth factors may be able to replace EGF in vivo. Also, no activity was seen against the SK-OV-3 ovarian cancer model, which is known to express other EGF receptor family members (although it is not clear whether these are absolutely required for growth in vivo). While substantial growth delays were seen in A431 and MCF-7 tumor models, the treated tumors remained approximately the same size throughout therapy, suggesting that the compounds are cytostatic rather than cytotoxic under these test conditions. It remains to be determined if more prolonged therapy has cytotoxic effects in vivo, resulting in net tumor cell kill.

The epidermal growth factor receptor (EGFR) has become an important enzyme target for cancer chemotherapy.[1−3] It plays a central role in growth signaling[4,5] and is overexpressed in a significant proportion of human tumors.[6,7] A major step forward in the development of EGFR-targeted drugs was the discovery of the 4-anilinoquinazoline class of compounds.[8−12] These are potent and selective inhibitors of the tyrosine kinase activity of the EGFR via competitive binding at the ATP site of the enzyme. Potent inhibition of the enzyme is associated with small lipophilic electron-withdrawing groups at the 3-position of the aniline ring and with electron-donating groups at the 6- and/or 7-positions of the quinazoline.[9−11] For example, **1** has an IC_{50} of 0.025 nM for inhibition of substrate phosphorylation by the isolated EGFR enzyme.[9] Two examples of this class (**2** and **3**) that exemplify these structure−activity relationships (SAR) are reported to be in clinical trial.[13,14]



We have recently shown[15] that related 4-(3-bromoanilino)pyrido[*d*]pyrimidines are also potent and selective inhibitors of both substrate phosphorylation by the isolated EGFR enzyme and its autophosphorylation in cells. The most potent subclass of this series of regioi-

S0022-2623(97)00641-9 CCC: $15.00
Published on Web 02/03/1998

somers was the pyrido[3,4-*d*]pyrimidines, and in particular the 6-methylamino derivative **5b** (PD 158780). This compound had an IC_{50} of 0.008 nM for inhibition of substrate phosphorylation,[15] compared with an IC_{50} of 7 nM for the corresponding 6-(methylamino)quinazoline analogue (**4**).[11]

A detailed study[16] of **5b** showed that it was a competitive inhibitor of the EGFR with respect to ATP and inhibited EGFR autophosphorylation in A431 human epidermoid carcinoma cells with an IC_{50} of 13 nM. While the onset of inhibition was immediate, recovery of receptor autophosphorylation was slow, requiring 8 h to complete. Compound **5b** was also active against other members of the EGFR family, with IC_{50}s of 49 and 52 nM respectively for inhibition of heregulin-stimulated autophosphorylation in SK-BR-3 and MDA-MB-453 breast carcinomas. SK-BR-3 cells express EGFR, *erbB*2, and *erbB*3, while MDA-MB-453 expresses *erbB*2, *erbB*3, and *erbB*4; heregulin is a specific ligand for *erbB4* but also binds and activates heterodimers of *erbB*3, *erbB*2, and *erbB*4.[16−18] Studies of the inhibition of clone formation in soft agar of a series of transformed fibroblasts and established human breast carcinoma lines confirmed that **5b** had good activity against members of the EGFR family, while not influencing the function of related tyrosine kinases.[16] The above properties of **5b** make it an attractive lead compound for further development.

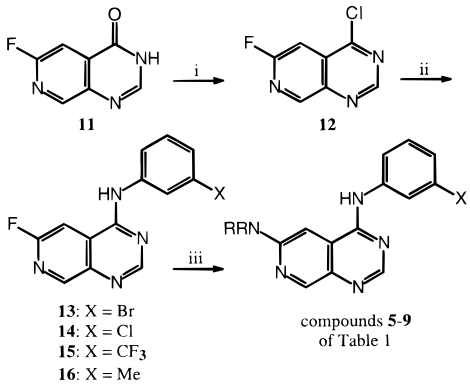
The broad SAR reported for the 4-anilinoquinazolines and pyrido[*d*]pyrimidines as EGFR inhibitors are consistent[19] with the compounds binding to the ATP site of the EGFR. In this model,[19] the N-1 atom accepts an H-bond from Met-769 and N-3 from the side chain of Thr-766 on strand 5 deep in the binding cleft, and the anilino side chain binds in an adjacent hydrophobic pocket. This pocket is exceptionally large in the EGFR, formed in part by three additional sulfur-containing amino acids (Cys-751, Met-769, and Met-742). This binding model suggests that the only positions on the model where substantial property-modulating groups can be placed are the 6- and 7-positions of the bicyclic chromophore, which are situated at the entrance of the adenine binding cleft. We have shown previously[20] that a variety of bulky side chains on the 7-position of pyrido-[4,3-*d*]pyrimidines are acceptable, but show a distinct SAR.

In this paper we report the synthesis and evaluation of a series of 6-substituted pyrido[3,4-*d*]pyrimidines, representing analogues of **5b** substituted on the 6-methylamino group. We also compare SAR for these compounds with that of the related 7-substituted pyrido-[4,3-*d*]pyrimidines for inhibition of isolated EGFR. Selected analogues were also evaluated for autophosphorylation in A431 cells and in vivo against a range of human tumor xenografts in nude mice.

Chemistry

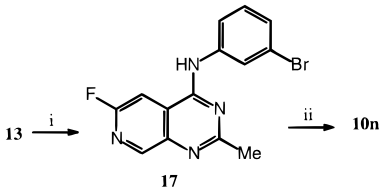
The 6-substituted 4-(phenylamino)pyrido[4,3-*d*]pyrimidines of Table 1 were prepared by the general method of Scheme 1. Activation of the known[21] 6-fluoropyrido-[3,4-*d*]pyrimidin-4(3*H*)-one (**11**) with $SOCl_2$/DMF gave 4-chloro-6-fluoropyrido[3,4-*d*]pyrimidine (**12**), and reaction of this with appropriate 3-substituted anilines gave the key intermediates **13**−**16**. Displacement of the 6-fluorine atom from these intermediates with amine nucleophiles was normally conducted in DMSO at 80−95 °C for 1−5 days (in a pressure vessel for the more volatile amines) followed by purification by direct recrystallization or chromatography (method A). With amino acid nucleophiles, it was necessary to preform the sodium salts prior to reaction (method B).[20]

Scheme 1[a]



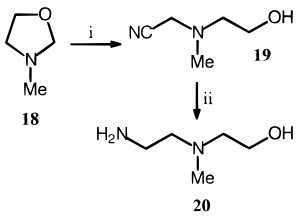
[a] (i) $SOCl_2$/dichloroethane/DMF/reflux/2.5 h; (ii) 3-X-aniline/2-propanol/reflux/45 min; (iii) RRNH/DMSO/85-95 °C/2 h−5 d.

Scheme 2[a]



[a] (i) $MeNO_2$/DBU/DMSO/25 °C/24 h; (ii) 4-(2-aminoethyl)morpholine/DMSO/80 °C/22 h.

Scheme 3[a]



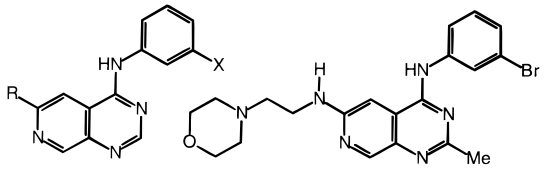
[a] (i) aqueous HCN; (ii) $LiAlH_4$.

The 2-methyl derivative **10n** was prepared by reaction of **13** with nitromethane to give **17** followed by fluorine displacement with 4-(2-aminomethyl)morpholine (Scheme 2). Most of the required amines were commercially available. 2-[(2-Aminoethyl)methylamino]ethanol was prepared by the general method of Kohn et al.[22] (Scheme 3).

Results and Discussion

The structures of the 6-substituted 4-(phenylamino)-pyrido[3,4-*d*]pyrimidines studied (**5**−**10**) are recorded in Table 1. Inhibition of tyrosine phosphorylation of a random tyrosine/glutamic acid copolymer (Sigma) by EGF-stimulated full-length EGFR enzyme isolated from A431 cells was measured by a filter binding assay.[8] At least two complete dose−response curves were determined for each compound, and averaged IC_{50}s are listed in Table 1. Most compounds were also evaluated for their ability to inhibit autophosphorylation of the EGF receptor in A431 human epidermoid carcinoma cells.

Table 1. Structural and Biological Properties of 6-Substituted 4-(Phenylamino)pyrido[3,4-*d*]pyrimidines



					IC$_{50}$ (nM)	
no.	R	X	mp (°C)	solubility[a] (nM)	enzyme[b]	autophos[c]
5a	NH_2	Br	ref 13		0.13	16
5b	NHMe	Br	ref 13	0.10	0.008	13
5c	NMe_2	Br	ref 13	0.06	0.006	21
5d	$NHCH_2CH_2OH$	Br	209−210	0.02	0.19	
5e	$N(Me)CH_2CH_2OH$	Br	236−237	0.11	0.22	
5f	$NHCH_2CH(OH)CH_2OH$	Br	186.5−188	0.21	0.18	
5 g	$N(Me)CH_2CH(OH)CH_2OH$	Br	159−160	0.18	0.56	
5h	$NH(CH_2)_2NMe_2$	Br	250−252	>48	1.1	43
5i	$NH(CH_2)_3NMe_2$	Br	160−161	30	1.2	16
5j	$NH(CH_2)_4NMe_2$	Br	176−177	38	1.8	19
5k	$NHCH_2CH(OH)CH_2NEt_2$	Br	189−192	36	4.6	28
5l	$NH(CH_2)_2N(Me)CH_2CH_2OH$	Br	182−189	>45	1.7	27
5m	$N(Me)(CH_2)_2NMe_2$	Br	260−262	39	8.1	46
5n	$NH(CH_2)_2$morpholinyl	Br	267 dec	10	0.65	7.9
5o	$NH(CH_2)_3$morpholinyl	Br	177−178	>44	1.0	5.9
5p	$NH(CH_2)_3$NMepiperazinyl	Br	182−183	32	3.9	8.9
5q	$NH(CH_2)_2N(CH_2CH_2OH)_2$	Br	210−211	36	0.93	71
5r	$NH(CH_2)_3N(CH_2CH_2OH)_2$	Br	205−207	20	0.35	230
5s	$NHCH_2$(3-pyridyl)	Br	260−262	12	1.5	13
5t	$NHCH_2CH_2$(2-pyridyl)	Br	170−171.5	17	1.2	8.1
5u	$NH(CH_2)_2$(4-imidazolyl)	Br	200−201	15	0.78	6.3
5v	$NH(CH_2)_3$(1-imidazolyl)	Br	195.5−197	23	1.7	9.1
5w	4-Mepiperazinyl	Br	219.5−222	2.5	6.4	102
5x	$NHCH_2COOH$	Br	234−239	5	0.28	
5y	$N(Me)CH_2COOH$	Br	225−227	44	0.44	
5z	$NH(CH_2)_2COOH$	Br	276−280	38	0.27	449
6b	NHMe	H	ref 13		9	
7b	NHMe	Cl	185.5−187		0.19	
8b	NHMe	CF_3	172−173		1.1	
9a	NH_2	Me	235.5−237	0.4	3.1	
9b	NHMe	Me	189−190		0.45	23
9c	NMe_2	Me	239−241	0.02	2.8	
9n	$NH(CH_2)_2$morpholinyl	Me	168−170	>40	1.5	20
9o	$NH(CH_2)_3$morpholinyl	Me	200−203	>35	1.8	
9u	$NH(CH_2)_2$(4-imidazolyl)	Me	207−209	>30	1.3	
10n	$NH(CH_2)_2$morpholinyl[d]	Br	>260		117	

[a] Solubility in water at 20 °C, determined by HPLC (see text). Values are for the hydrochloride or dihydrochloride salt form of amines and the sodium salt form of acids. [b] IC$_{50}$, concentration of drug (nM) to inhibit the phosphorylation of a random tyrosine/glutamic acid 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] 2-Me analogue.

Compound aqueous solubilities were determined by HPLC following sonication for 30 min at 20 °C. Lactate buffer (0.05 M) was used for compounds with neutral side chains and water for hydrochloride salts of amines and sodium salts of acids.

The initial set of 6-methylamino compounds (**6b**, **7b**, **8b**, and **9b**) was prepared to compare with **5b** and evaluate SAR for the anilino side-chain substituent. A number of small lipophilic substituents have been used at the 3′-position in anilinoquinazolines[9,12−14] and corresponding pyrido[4,3-*d*]pyrimidines,[23] including Cl, CF_3, Me, and ethynyl. We have previously shown[11] that the 3′-Br derivative was the most potent enzyme-inhibitory analogue in the anilinoquinazoline series, and this proved also to be the case with the pyrido[3,4-*d*]-pyrimidines. The 3′-Br analogue **5b** (IC$_{50}$ 0.008 nM) proved by far to be the most potent inhibitor of the isolated enzyme, and we focused mainly on this substituent in developing the 6-substituted series, although a small number of 3-Me analogues were also prepared for comparison purposes.

Compounds **5d**−**g**, bearing hydroxyl substituents, although considerably less potent than the parent **5b** against the isolated enzyme, retained acceptable levels of inhibition (IC$_{50}$s <1 nM). However, they did not show improved aqueous solubility over **5b**. In contrast, all but one of the basic analogues **5h**−**w** had aqueous solubilities of ≥10 mM; the exception was **5w**, with a rigid, directly attached piperazine substituent. All of the weak bases were evaluated for inhibition of isolated enzyme and of autophosphorylation. Compounds **5h**,**m** compared the utility of a secondary versus tertiary amine linkage; the tertiary amine **5m** was less effective against isolated enzyme, but no difference was seen in the autophosphorylation assay. Because of this result, and a more extensive evaluation of this aspect (which

showed no advantage) in the related pyrido[4,3-*d*]-pyrimidines,[20] tertiary amine linkages were not investigated further.

For the 14 compounds with NH-linked amine side chains (**5h**−**l**, **5n**−**v**), no clear SAR emerges for inhibition of substrate phosphorylation by the isolated enzyme (Table 1). These 6-substituents vary in amine base strength (from about pK_a 7 to 10), the distance of the cationic center from the chromophore (from 3 to 5 atoms), and the overall lipophilicity of the side chain (containing from one to two additional OH groups). However, nearly all of have IC_{50} values of around 1 nM, varying by less than 10-fold (except for two outliers, **5k**,**p**, by less than 5-fold). There is no variation with positioning of the cation (**5h**−**j**), with side-chain lipophilicity (for example, **5h**,**q**), or with base strength (several examples). This is in contrast to the 7-substitituted pyrido[4,3-*d*]pyrimidines,[20] where IC_{50}s between analogues with cationic side chains varied by about 100-fold, improving with distance from the chromophore and for weak bases. While the modeling studies[19] show that both 6- and 7-substituents are in an area of bulk tolerance in the binding site, at the entrance of the ATP pocket, the above results suggest that 6-substituents are in a more favorable environment.

There were clear SARs for the ability of the basic compounds to inhibit EGFR autophosphorylation in A431 cells (Table 1). The hydrophilic OH-containing analogues, and especially the diols **5q**,**r**, were relatively inactive, despite high potency against the isolated enzyme. This is almost certainly due to poor cellular uptake characteristics. With the exception of the $(CH_2)_2$-NMe_2 analogue **5h**, where the cationic charge is positioned closest to the chromophore, all the other NH-linked analogues were at least as potent as the parent **5b**. Overall, the weaker bases (e.g., **5u**) appeared to show better activity. A small series of analogous 3′-methyl derivatives with amine side chains was also studied (compounds **9n**,**o**,**u**) but these were less active than their 3′-Br analogues in both assays, as shown previously[12,23−25] in several series of related EGFR inhibitors. The 2-methyl compound **10n** was prepared to check bulk tolerance at this position, but it showed very poor activity, as suggested both from previous studies with 4-anilinoquinazolines[11] and from the binding model.[19]

Three anionic derivatives (**5x**−**z**) were also examined. These were more potent than any of the amine analogues against the isolated enzyme, but **5z** showed very poor activity in the cell-based autophosphorylation assay. A similar result was seen for the 7-substituted pyrido[4,3-*d*]pyrimidines[20] and is again attributed to poor cellular uptake.

From the above results, three compounds (**5h**,**k**, **n**), which represent the range of structural variations studied, were selected for comparison with the parent **5b** in xenograft assays in vivo. They all had much better aqueous solubility than **5b** and showed acceptable activity in the two screening assays (IC_{50}s for inhibition of the isolated enzyme of ∼1 nM and IC_{50}s comparable to that of the parent **5b** for inhibition of autophosphorylation). Like **5b**,[16] all showed an ability to shut down both EGF and heregulin-stimulated receptor autophosphorylation in cells (Table 2), suggesting they are broad-spectrum inhibitors across the *erbB* family. At the same time, as also shown previously for **5b**,[16] these compounds had little or no activity against a series of other receptor tyrosine kinases (Table 3), indicating a high degree of specificity for the *erbB* family.

Table 2. Inhibition (IC_{50} Values, μM) of EGF-Dependent Verus Heregulin-Dependent Tyrosine Autophosphorylation

no.	EGF[a]	heregulin[b]
5b	13	52
5h	43	379
5k	28	1100
5n	7.9	219

[a] EGF-dependent receptor autophosphorylation in A431 cells. [b] Heregulin-dependent receptor autophosphorylation in MDA-MB-453 cells.

Table 3. Selectivity of Inhibition of Isolated EGFR Over Other Receptor Tyrosine Kinases by Selected Analogues (IC_{50} Values, μM)

no.	EGFR[a]	c-Src[b]	FGFR[c]	PDGFR[d]	IR[e]
5h	0.0011	>50 (42%) [f]	>50 (41%)	>50 (14%)	>50 (0%)
5k	0.0046	>50 (26%)	>50 (24%)	>50 (12%)	>50 (0%)
5n	0.0007	>50 (14%)	>50 (39%)	>50 (17%)	>50 (12%)

[a] See footnote b, Table 1. [b] For conditions, see ref 36. [c] Fibroblast growth factor receptor; for conditions, see ref 36. [d] Platelet-derived growth factor receptor; for conditions, see ref 36. [e] Insulin receptor; for conditions, see ref 36. [f] Percentage inhibition at 50 μM.

These compounds were evaluated in four xenograft tumor model systems in nude mice (Table 4), and the 3′-methyl analogue **9b** was also studied in the EGFR line. The A431 epidermoid xenograft was selected based on its in vitro mitogenic responsiveness to EGF and 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.[26] The MCF-7 breast and SK-OV-3 ovarian tumor models were selected based on their in vivo expression of other EGF receptor family members.

Compound **5b** had the best therapeutic effect against the A431 epidermoid carcinoma when administered either intraperitoneally or orally (Table 4) compared to compounds **5h**,**k**,**n** at equitoxic doses. The latter three compounds produced only marginal responses in this model. All three compounds (**5b**,**h**,**k**) produced measurable, significant effects against a mouse fibroblast transfected with human EGFR. The antitumor effects of these compounds were lost when the dose route was changed from intraperitoneal to peroral. Compounds **5n** and **9b** were also evaluated against this tumor model but were ineffective.

Neither compound **5b** nor **5n** was effective against the SK-OV-3 ovarian xenograft. This was unexpected, since this cell line overexpresses *erbB*2. The lack of effectiveness may indicate that although this cell line overexpresses *erbB*2 it may not be dependent on this receptor for growth in vivo. Compounds **5b**,**n** produced a significant therapeutic effect against the estrogen-dependent MCF-7 breast carcinoma at equitoxic dose levels, whereas **5k** was ineffective against this tumor model. Compound **5b** was the most active against the human tumor xenografts A431 and MCF-7 when evaluated at the equitoxic doses reported in the table.

Table 4. In Vivo Activity of Selected Analogues against Tumor Xenografts in Nude Mice

no.	tumor[a]	dose (mg/kg)	schedule[b]	weight change (g)	T/C(%) on last therapy day[c]	T−C (days)[d]	log net cell kill[e]
5b	A431	75	ip, b.i.d. days 7−21	−0.8	41	8.4[g]	−0.3
	A431	100	po, days 7−21	−1.3	38	16.0	+0.1
	EGFR	38	ip, b.i.d. days 1−15	−0.1	38	3.7[g]	−1.0
	EGFR	400[f]	po, days 1−15	+	67	1.2	−1.3
	MCF-7	50	ip, b.i.d. days 1−15	+	27	15.2[g]	+0.1
	MCF-7	25	ip, b.i.d. days 1−15	−0.2	14	11.2[g]	−0.1
	SK-OV-3	30	ip, b.i.d. days 10−14, 17−21, 24−28	−1.6	67	0.8	−0.7
5h	A431	25	ip, b.i.d. days 7−21	−1.4	66	4.7	−0.5
	EGFR	25	ip, b.i.d. days 1−15	−1.1	43	3.3[g]	−1.0
	EGFR	200	po, days 1−15	+	47	1.1	−1.3
5k	A431	25	ip, b.i.d. days 7−21	−0.7	62	5.0	−0.5
	EGFR	25	ip, b.i.d. days 1−15	−0.9	44	5.0[g]	−0.9
	EGFR	200	po, days 1−15	+	94	0	
	MCF-7	25	ip, b.i.d. days 1−15	−0.3	77	3.3	−0.6
5n	A431	12	ip, b.i.d. days 7−21	−0.2	93	1.9	−0.7
	A431	25	ip, b.i.d. days 13−26	−1.1	98	2.1	−0.3
	EGFR	25	ip, b.i.d. days 1−15	−1.3	77	1.2	−1.2
	EGFR	400[f]	po, days 1−15	−2.0	15	2.8	−1.1
	MCF-7	25	ip, b.i.d. days 1−15	+	34	11.7[g]	−0.1
	SKOV-3	25	ip, b.i.d. days 10−14, 17−21, 24−28	−2.1	100	0	
9b	EGFR	38	ip, b.i.d. days 1−15	−0.9	68	2.0	−1.2

[a] The indicated tumor fragments were implanted sc into the right axilla of mice on day 0. [b] Compounds were administered intraperitoneally or orally on the indicated schedules. The maximum tolerated dose (LD_{10}) from a complete dose−response is shown for individual experiments. Reporting the maximum tolerated dose allows comparison of the antitumor effectiveness of test compounds at equitoxic dose levels. [c] Ratio of median treated tumor mass/median control tumor mass × 100%. [d] The difference in days for the treated (T) and control (C) tumors to reach 750 mg. [e] The net reduction in tumor burden, in log, between the first and last treatments. [f] Highest dose tested. [g] Significantly different from control (*t*-test, $p < 0.05$).

Compound **5n** was active only against the MCF-7 tumor model. In the A431 and MCF-7 models the tumors treated with equitoxic doses of these compounds did not increase substantially in size over the course of therapy but resumed growth at the cessation of therapy.

Conclusions

This study was designed to determine the effects of water-soluble groups attached at the 6-NHMe substituent of the extremely potent EGFR inhibitor **5b** (PD 158780). A small series of 3′-substituted analogues of **5n** confirmed that a 3′-bromo atom provided the most potent derivative, and this was primarily used in the main study. While compounds bearing neutral hydroxylated substituents at the 6-position retained relatively good inhibitory potencies, aqueous solubility was not improved, while anionic substituents resulted in poor cellular uptake (as judged by relatively low potencies in the autophosphorylation assay). The most suitable substituents were weak bases attached through a secondary amine linkage; the corresponding compounds had aqueous solubilities of ≥10 mM and IC_{50}s for inhibition of the isolated enzyme of ca. 1 nM. No clear SAR could be discerned for amine base strength or the distance of the cationic center from the chromophore, suggesting that 6-substituents are in a favorable area of bulk tolerance in the enzyme binding site. More distinct SAR emerged for the ability of the compounds to inhibit EGFR autophosphorylation in A431 cells, where analogues bearing lipophilic weak bases were the best.

Three analogues (**5h**,**k**,**n**), with potent activity against the isolated enzyme and possessing soluble basic side chains where the above important parameters of lipophilicity and base strength were varied, were evaluated for antitumor effectiveness against four in vivo tumor model systems, along with the parent **5b** and a 3′-Me analogue (**9b**). Meaningful in vivo activity against human xenografts was observed only in two model systems: the estrogen-dependent MCF-7 breast and A431 epidermoid tumors. The small but significant in vivo growth delay effects of these compounds against the EGFR tumor model are reflective of the fact that this manufactured cell line doubles in volume each day and hence progresses very rapidly. These results may also indicate that while this cell line requires EGF for clone formation in soft agar, other growth factors may be able to replace EGF in vivo.

No activity was seen for compounds **5b**,**n** against the SK-OV-3 ovarian tumor. While both the MCF-7 and SK-OV-3 tumors are known to express other EGF receptor family members in vivo, these may not be absolutely required for growth in vivo. However, two of the three compounds evaluated against MCF-7 (**5b**,**n**) showed significant antitumor effects. These two compounds also had the lowest relative IC_{50} values for the inhibition of heregulin-dependent tyrosine phosphorylation in vitro (Table 2). It is notable that in the human tumor xenografts A431 and MCF-7, where responses were observed, the treated tumors remained approximately the same size throughout therapy, suggesting that these compounds are cytostatic rather than cytotoxic under these test conditions. It remains to be determined if more prolonged therapy would have cytotoxic effects in vivo, resulting in high positive net cell kill values.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. 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. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution. HPLC was

carried out using a Bondclone 10 C18 column, with a Phillips PU4100M gradient elution pump and a Phillips PU 4120 diode array detector, eluting with the appropriate ratios of 80% acetonitrile/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).

2-[(2-Aminoethyl)methylamino]ethanol (20), Scheme 3. Condensation of 3-methyloxazolidine (**18**) and aqueous HCN as reported[22] gave [*N*-(2-hydroxyethyl)methylamino]-acetonitrile (**19**) (93%) as an oil: bp 70−72 °C/0.03 mmHg (lit.[27] bp 62−63 °C/0.05 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.70−3.64 (br m, with D_2O wash collapses to t, J = 5.3 Hz at δ 3.67, 2 H), 3.61 (s, 2 H), 2.69 (t, J = 5.3 Hz, 2 H), 2.43 (s, 3 H), 2.19 (br s, exchanges with D_2O); CIMS *m*/*z* (relative intensity) 115 (MH^+, 46), 88 (100).

Reduction of **19** with $LiAlH_4$ gave **20** as an oil: bp 81−82 °C/0.42 mmHg (lit.[28] bp 115−117 °C/16 mmHg, lit.[29] bp 103−104 °C/8 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.61 (t, J = 5.3 Hz, 2 H), 2.81 (t, J = 5.8 Hz, 2 H), 2.55 (t, J = 5.5 Hz, 2 H), 2.50 (t, J = 6.3 Hz, 2 H), 2.35 (br s, 3 H), 2.29 (s, 3 H); CIMS *m*/*z* (relative intensity) 119 (MH^+, 100), 102 (97), 88 (91).

4-[(3-Bromophenyl)amino]-6-fluoropyrido[3,4-*d*]pyrimidine (13), Scheme 1. A stirred suspension of 6-fluoropyrido[3,4-*d*]pyrimidin-4(3*H*)-one[21] (**11**) (30.0 g, 182 mmol) in 1,2-dichloroethane (182 mL) was treated successively with $SOCl_2$ (182 mL) and then ca. 1 mL of DMF. The mixture was heated at reflux for 2.5 h, then concentrated to a solid, and coevaporated twice with 1,2-dichloroethane. The residue was dissolved in CH_2Cl_2 and filtered through a short pad of silica gel, eluting with CH_2Cl_2 to give 4-chloro-6-fluoropyrido[3,4-*d*]-pyrimidine (**12**) (30.5 g, 91%). A sample was crystallized from *tert*-butyl methyl ether: mp 75−76 °C; ^{1}H NMR ($CDCl_3$) δ 9.29 (s, 1 H, H-2), 9.16 (s, 1 H, H-8), 7.65 (dd, J = 0.7, 2.0 Hz, 1 H, H-5); ^{19}F NMR δ −68.9 (s); ^{13}C NMR δ 162.1 (d, J_{C-F} = 7 Hz), 161.8 (d, J_{C-F} = 242 Hz), 154.1 (d, J_{C-F} = 2 Hz), 153.4 (d, J_{C-F} = 15.3 Hz), 143.7 (d, J_{C-F} = 3 Hz), 130.7 (d, J_{C-F} = 9 Hz), 100.9 (d, J_{C-F} = 39.7 Hz). Anal. ($C_7H_3N_3ClF$) C, H, N.

A mechanically stirred solution of **12** (30.0 g, 163 mmol) and 3-bromoaniline (33.75 g, 196 mmol) in 2-propanol (400 mL) was heated at reflux for 45 min. The resulting suspension was concentrated to ca. 150 mL, and the resulting precipitate was collected, washed successively with 2% aqueous NaOH to neutral pH, water, and 2-propanol, and dried over P_2O_5 to give **13** (49.1 g, 94%): mp (2-propanol) 224−226 °C (lit.[15] mp 219.5−221 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 10.06 (br s, 1 H, NH), 8.94 (s, 1 H, H-8), 8.73 (s, 1 H, H-2), 8.23 (br s, 2 H, H-5,2′), 7.90 (br d, J = 7.5 Hz, 1 H, H-6′), 7.39 (t, J = 7.8 Hz, 1 H, H-5′), 7.35 (d, J = 8.0 Hz, 1 H, H-4′); ^{19}F NMR δ −74.12 (s); ^{13}C NMR δ 159.8 (d, J_{C-F} = 233 Hz, C-6), 156.6 (d, J_{C-F} = 5 Hz), 154.9 (C-2), 150.7 (d, J_{C-F} = 15 Hz, C-8), 142.8, 140.0, 130.5, 126.7, 124.1, 123.2 (d, J_{C-F} = 9 Hz), 121.2, 120.6, 99.6 (d, J_{C-F} = 40 Hz, C-5); CIMS *m*/*z* (relative intensity) 321 (MH + 2^+, 65), 319 (MH^+, 100).

The following compounds were prepared similarly.

4-[(3-Chlorophenyl)amino]-6-fluoropyrido[3,4-*d*]pyrimidine (14): from **12** and 3-chloroaniline (89%); mp (MeOH/H_2O) 224−225 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 8.97 (s, 1 H, H-8), 8.76 (s, 1 H, H-5), 8.28 (s, 1 H, H-2), 8.14 (t, J = 2.0 Hz, 1 H, H-2′), 8.26 (dd, J = 8.2, 1.3 Hz, 1 H, H-6′), 7.47 (t, J = 8.1 Hz, 1 H, H-5′), 7.24 (dd, J = 8.0, 1.4 Hz, 1 H, H-4′). Anal. ($C_{14}H_{12}ClN_5$) C, H, N.

6-Fluoro-4-[[3-(trifluoromethyl)phenyl]amino]pyrido-[3,4-*d*]pyrimidine (15): from **12** and 3-(trifluoromethyl)-aniline (95%); mp (MeOH/H_2O) 209−211 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 10.25 (s, 1 H, NH), 8.99 (s, 1 H, H-8), 8.78 (s, 1 H, H-5), 8.35 (s, 1 H, H-2), 8.30 (s, 1 H, H-2′), 8.26 (d, J = 8.3 Hz, 1 H, H-6′), 7.68 (t, J = 8.0 Hz, 1 H, H-5′), 7.53 (d, J = 7.7 Hz, 1 H, H-4′). Anal. ($C_{15}H_{12}F_3N_5$) C, H, N.

6-Fluoro-4-[(3-methylphenyl)amino]pyrido[3,4-*d*]pyrimidine (16): from **12** and *m*-toluidine (92%); mp (MeOH/H_2O) 190−192 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.99 (s, 1 H, NH), 8.93 (s, 1 H, H-8), 8.68 (d, J_{H-F} = 1.8 Hz, 1 H, H-5), 8.29 (s, 1 H, H-2), 7.72−7.68 (m, 2 H, H-2′,6′), 7.32 (t, J = 8.0 Hz, 1 H, H-5′), 7.02 (d, J = 7.4 Hz, 1 H, H-4′), 2.36 (s, 3 H, CH_3). Anal. ($C_{14}H_{11}FN_4$) C, H, N.

4-[(3-Bromophenyl)amino]-6-(methylamino)pyrido[3,4-*d*]pyrimidine (5b), General Method A of Scheme 1. A 2-L stainless steel reactor was flushed with dry N_2 and charged with **13** (35.0 g, 108 mmol) and anhydrous $MeNH_2$ (57.5 g, 1.85 mol) in DMSO (1 L). The reactor was sealed and heated at 80 °C for 24 h and then cooled. After venting off the excess $MeNH_2$, the mixture was concentrated to ca. 500 mL and poured slowly into 2.5 L of water with vigorous stirring. The precipitate was collected, washed well with water, and dried over P_2O_5 to afford **5b**: mp (2-propanol) 181−183 °C (lit.[15] mp 172−173 °C from MeOH/H_2O). Total yield after chromatography of the mother liquor on silica gel: 32.5 g (91%).

The following compounds were prepared similarly.

4-[(3-Bromophenyl)amino]-6-[(2-hydroxyethyl)amino]-pyrido[3,4-*d*]pyrimidine (5d): from **13** and ethanolamine (35% yield after two recrystallizations from MeOH); mp 209−210 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.20 (br s, 1 H, H-2′), 7.91 (br d, J = 8.0 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.4 Hz, 1 H, H-4′), 7.15 (s, 1 H, H-5), 6.66 (t, J = 5.7 Hz, 1 H, NH), 4.80 (t, J = 5.3 Hz, 1 H, OH), 3.65 (q, J = 5.8 Hz, 2 H, CH_2), 3.38 (q, J = 6.1 Hz, 2 H, CH_2). Anal. ($C_{15}H_{14}BrN_5$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[*N*-(2-hydroxyethyl)-*N*-methylamino]pyrido[3,4-*d*]pyrimidine (5e). A mixture of **13** (0.20 g, 0.63 mmol) and 2-(methylamino)ethanol (2.4 g, 31 mmol, 50 equiv) in EtOH (50 mL) was heated at 95 °C for 18 h in a sealed pressure vessel. The solvent was removed under reduced pressure, and the residue was triturated with water and recrystallized from MeOH to give **5e** (0.21 g, 89%): mp 236−237 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (s, 1 H, NH), 8.82 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.93 (br d, J = 8.0 Hz, 1 H, H-6′), 7.38 (t, J = 8.0 Hz, 1 H, H-5′), 7.33 (br d, J = 8.4 Hz, 1 H, H-4′), 7.24 (s, 1 H, H-5), 4.73 (t, J = 5.3 Hz, 1 H, OH), 3.76 (t, J = 6.1 Hz, 2 H, CH_2), 3.63 (dd, J = 6.1, 5.6 Hz, 2 H, CH_2), 3.19 (s, 3 H, CH_3). Anal. ($C_{16}H_{16}BrN_5O$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[(2,3-dihydroxypropyl)-amino]pyrido[3,4-*d*]pyrimidine (5f): from **13** and 2,3-dihydroxypropylamine (42%); mp (MeOH) 186.5−188 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.74 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.21 (br s, 1 H, H-2′), 7.91 (br d, J = 7.9 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.4 Hz, 1 H, H-4′), 7.16 (s, 1 H, H-5), 6.46 (t, J = 5.5 Hz, 1 H, NH), 4.93 (d, J = 4.9 Hz, 1 H, OH), 4.68 (t, J = 5.5 Hz, 1 H, OH), 3.77 (sextet, J = 5.5 Hz, 1 H, CH), 3.47−3.39 (m, 2 H, CH_2), 3.27−3.20 (m, 2 H, CH_2). Anal. ($C_{16}H_{16}BrN_5O_2 \cdot 0.5H_2O$) C, H; N: found, 18.1; calcd, 18.5.

4-[(3-Bromophenyl)amino]-6-[*N*-(2,3-dihydroxypropyl)-*N*-methylamino]pyrido[3,4-*d*]pyrimidine (5g): from **13** and 2,3-dihydroxypropyl-*N*-methylamine[20] (72%); mp (MeOH) 159−160 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H, NH), 8.82 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.18 (t, J = 1.9 Hz, 1 H, H-2′), 7.93 (br d, J = 8.2 Hz, 1 H, H-6′), 7.39 (t, J = 8.0 Hz, 1 H, H-5′), 7.33 (br d, J = 8.4 Hz, 1 H, H-4′), 7.22 (s, 1 H, H-5), 4.75 (d, J = 4.9 Hz, 1 H, OH), 4.59 (t, J = 5.7 Hz, 1 H, OH), 3.84−3.79 (m, 2 H, CH_2), 3.59 (dd, J = 8.7, 6.7 Hz, 1 H, CH), 3.41−3.35 (m, 2 H, CH_2), 3.19 (s, 3 H, CH_3). Anal. ($C_{17}H_{18}BrN_5O_2$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[2-(dimethylamino)-ethyl]amino]pyrido[3,4-*d*]pyrimidine (5h): from **13** and 2-(dimethylamino)ethylamine (63%); mp (CH_2Cl_2) 114−118 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.69 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.22 (t, J = 1.9 Hz, 1 H, H-2′), 7.91 (br d, J = 8.0 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.4 Hz, 1 H, H-4′), 7.15 (s, 1 H, H-5), 6.50 (t, J = 5.4 Hz, 1 H, NH), 3.38 (dd, J = 6.6, 5.7 Hz, 2 H, CH_2), 2.53 (t, J = 6.6 Hz, 2 H, CH_2), 2.23 (s, 6 H, CH_3). Dihydrochloride salt (from EtOH): mp 250−252 °C. Anal. ($C_{17}H_{19}BrN_6 \cdot 2HCl$) C, H, N, Cl.

4-[(3-Bromophenyl)amino]-6-[[3-(dimethylamino)propyl]amino]pyrido[3,4-*d*]pyrimidine (5i): from **13** and

3-(dimethylamino)propylamine (80%); mp (CH_2Cl_2/hexane) 160−161 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.20 (t, J = 1.8 Hz, 1 H, H-2′), 7.91 (br d, J = 8.2 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.4 Hz, 1 H, H-4′), 7.08 (s, 1 H, H-5), 6.84 (t, J = 5.4 Hz, 1 H, NH), 3.29 (q, J = 6.2 Hz, 2 H, CH_2), 2.35 (t, J = 6.9 Hz, 2 H, CH_2), 2.15 (s, 6 H, CH_3), 1.78 (pentet, J = 6.9 Hz, 2 H, CH_2). Anal. ($C_{18}H_{21}BrN_6$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[4-(dimethylamino)butyl]amino]pyrido[3,4-*d*]pyrimidine (5j): from **13** and 4-(dimethylamino)butylamine (98%); mp (CH_2Cl_2/hexane) 176−177 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.68 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.39 (s, 1 H, H-2), 8.21 (t, J = 1.8 Hz, 1 H, H-2′), 7.92 (br d, J = 8.3 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (dd, J = 8.4, 1.2 Hz, 1 H, H-4′), 7.06 (s, 1 H, H-5), 6.87 (t, J = 5.6 Hz, 1 H, NH), 3.27 (q, J = 6.4 Hz, 2 H, CH_2), 2.24 (t, J = 7.1 Hz, 2 H, CH_2), 2.12 (s, 6 H, CH_3), 1.65 (pentet, J = 7.1 Hz, 2 H, CH_2), 1.53 (pentet, J = 7.2 Hz, 2 H, CH_2). Anal. ($C_{19}H_{23}BrN_6$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[3-(diethylamino)-2-hydroxypropyl]amino]pyrido[3,4-*d*]pyrimidine (5k). A solution of **13** (5.74 g, 18 mmol) and 1-amino-3-(diethylamino)-2-propanol[30] (26.3 g, 180 mmol) in DMSO (27 mL) was heated at 80 °C under N_2 for 23 h. The DMSO was evaporated under reduced pressure (0.1 mm) at 100 °C, and the residue was purified by flash chromatography on silica gel. Elution with a gradient of MeOH in EtOAc containing 2% Et_3N and pooling of appropriate fractions gave **5k** (4.87 g). Rechromatography of the mother liquor gave an additional 0.48 g (total yield 67%): mp (dihydrochloride salt from ^{i}PrOH/CH_2Cl_2/HCl) (EtOAc) 189−192 °C; ^{1}H NMR [free base, $(CD_3)_2SO$] δ 9.72 (s, exchanges with D_2O, 1 H), 8.75 (s, 1 H), 8.41 (s, 1 H), 8.21 (t, J = 1.9 Hz, 1 H), 7.91 (d, J = 7.0 Hz, 1 H), 7.39−7.29 (m, 2 H), 7.14 (s, 1 H), 6.63 (t, J = 5.3 Hz, exchanges with D_2O, 1 H), 4.77 (d, J = 4.3 Hz, exchanges with D_2O, 1 H), 3.87−3.83 (m, 1 H), 3.39−3.28 (m, 2 H), 2.58−2.41 (m, 6 H), 0.96 (t, J = 7.0 Hz, 6 H); CIMS m/z (relative intensity) 445 (MH^+, 13), 447 (13). Anal. ($C_{20}H_{25}BrN_6O\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[2-[*N*-(2-hydroxyethyl)-methylamino]ethyl]amino]pyrido[3,4-*d*]pyrimidine (5l). Reaction of **13** with 2-[(2-aminoethyl)methylamino]ethanol (**20**), followed by column chromatography, gave **5l** (60% as the dihydrochloride salt): mp (CH_2Cl_2/2-propanol) 182−189 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.63 (br s, 1 H, exchanges with D_2O), 10.11 (br s, 1 H, exchanges with D_2O), 8.92 (s, 1 H), 8.72 (s, 1 H), 8.21 (s, 1 H), 7.94 (d overlapping s, 2 H, with D_2O wash collapses to δ 7.85, d, J = 8.2 Hz, 1 H, δ 7.68, s, 1 H), 7.60−7.40 (br s overlapping m, 3 H, with D_2O wash collapses to d, 2 H), 3.84−3.75 (m, 4 H), 3.53−3.40 (m, 1 H), 3.40−3.27 (m, 2 H), 3.26−3.16 (m, 1 H), 2.90 (d, J = 4.8 Hz, 3 H, with D_2O wash collapses to s); CIMS m/z (relative intensity) 417 (MH^+, 26), 419 (25). Anal. ($C_{18}H_{21}BrN_6O\cdot 2HCl\cdot 0.5\ H_2O$) C, H, N, Cl.

A higher R_f component, eluted from the column with EtOAc/MeOH/Et_3N (90:10:1) (29 mg), was identified as **5e**, resulting from nucleophilic addition of the tertiary amine of the side chain followed by extrusion of aziridine.

4-[(3-Bromophenyl)amino]-6-[*N*-[2-(dimethylamino)-ethyl]-*N*-methylamino]pyrido[3,4-*d*]pyrimidine (5m): from **13** and 2-(dimethylamino)-*N*-methylethylamine (72%); ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.83 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.92 (br d, J = 7.9 Hz, 1 H, H-6′), 7.38 (t, J = 8.0 Hz, 1 H, H-5′), 7.33 (br d, J = 8.0 Hz, 1 H, H-4′), 7.21 (s, 1 H, H-5), 3.80 (t, J = 6.8 Hz, 2 H, CH_2), 3.14 (s, 3 H, CH_3), 2.45 (t, J = 6.8 Hz, 2 H, CH_2), 2.19 (s, 6 H, CH_3). Dihydrochloride salt (from EtOH): mp 260−262 °C. Anal. ($C_{18}H_{21}BrN_6\cdot 2HCl$) C, H, N, Cl.

4-[(3-Bromophenyl)amino]-6-[[2-(4-morpholino)ethyl]-amino]pyrido[3,4-*d*]pyrimidine (5n). A mixture of **13** (0.20 g, 0.63 mmol) and 4-(2-aminoethyl)morpholine (4.1 g, 31 mmol, 50 equiv) in DMSO (40 mL) was heated at 95 °C for 18 h. Most of the DMSO was removed under reduced pressure at 80 °C, and the residue was diluted with water and extracted into EtOAc. After being washed twice with water, the organic layer was dried (Na_2SO_4), and the solvent was removed under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/MeOH (99:1), gave **5n** (0.19 g, 71% yield): mp (MeOH) 185−187 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.69 (s, 1 H, exchangeable with D_2O, NH), 8.76 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.21 (t, J = 1.9 Hz, 1 H, H-2), 7.91 (br d, J = 8.1 Hz, 1 H, H-6), 7.37 (t, J = 8.0 Hz, 1 H, H-5), 7.31 (br d, J = 7.9 Hz, 1 H, H-4), 7.15 (s, 1 H, H-5), 6.55 (t, J = 5.5 Hz, 1 H, exchangeable with D_2O, NH), 3.60 (t, J = 4.6 Hz, 4 H, CH_2O), 3.41 (dd, J = 6.5, 5.9 Hz, 2 H, CH_2N), 2.60 (t, J = 6.7 Hz, 2 H, CH_2N), 2.46 (m, 4 H, CH_2N). Anal. ($C_{19}H_{21}BrN_6O$) C, H, N. Dihydrochloride salt: mp (MeOH/EtOH) 267 °C dec. Anal. ($C_{19}H_{21}BrN_6O\cdot 2HCl$) C, H, N, Cl.

4-[(3-Bromophenyl)amino]-6-[[3-(4-morpholino)propyl]amino]pyrido[3,4-*d*]pyrimidine (5o): from **13** and 4-(3-aminopropyl)morpholine (87%); mp (MeOH/H_2O) 177−178 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.91 (ddd, J = 8.0, 1.8, 1.2 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (dd, J = 8.0, 1.2 Hz, 1 H, H-4′), 7.08 (s, 1 H, H-5), 6.86 (t, J = 5.5 Hz, 1 H, NH), 3.58 (t, J = 4.6 Hz, 4 H, CH_2), 3.31 (q, J = 6.4 Hz, 2 H, CH_2), 2.41 (t, J = 7.0 Hz, 2 H, CH_2), 2.36 (br m, 4 H, CH_2), 1.81 (pentet, J = 6.9 Hz, 2 H, CH_2). Anal. ($C_{20}H_{23}BrN_6O$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[3-(4-methyl-1-piperazinyl)propyl]amino]pyrido[3,4-*d*]pyrimidine (5p): from **13** and 3-(4-methylpiperazyl)propylamine (83%); mp (CH_2Cl_2/hexane) 182−183 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.92 (br d, J = 8.2 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (dd, J = 8.3, 1.2 Hz, 1 H, H-4′), 7.07 (s, 1 H, H-5), 6.85 (t, J = 5.5 Hz, 1 H, NH), 3.30 (q, J = 6.3 Hz, 2 H, CH_2), 2.40 (t, J = 7.0 Hz, 2 H, CH_2), 2.33 (br m, 4 H, CH_2), 2.13 (s, 3 H, CH_3), 1.79 (pentet, J = 7.2 Hz, 2 H, CH_2). Anal. ($C_{21}H_{26}BrN_7$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[2-[*N*,*N*-bis(2-hydroxyethyl)amino]ethyl]amino]pyrido[3,4-*d*]pyrimidine (5q): from **13** and *N*,*N*-bis(2-hydroxyethyl)ethylamine (41%); mp (MeOH) 210−211 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.22 (br s, 1 H, H-2′), 7.92 (br d, J = 8.1 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.0 Hz, 1 H, H-4′), 7.14 (s, 1 H, H-5), 6.59 (t, J = 5.4 Hz, 1 H, NH), 4.44 (t, J = 5.4 Hz, 2 H, OH), 3.46 (dd, J = 5.9, 5.7 Hz, 4 H, CH_2), 3.34 (q, J = 6.2 Hz, 2 H, CH_2), 2.78 (t, J = 6.3 Hz, 2 H, CH_2), 2.62 (t, J = 6.1 Hz, 4 H, CH_2). Anal. ($C_{19}H_{23}BrN_6O_2$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[3-[*N*,*N*-bis(2-hydroxyethyl)amino]propyl]amino]pyrido[3,4-*d*]pyrimidine (5r): from **13** and *N*,*N*-bis(2-hydroxyethyl)propylamine (48%); ^{1}H NMR [$(CD_3)_2SO$] δ 9.68 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.21 (t, J = 1.8 Hz, 1 H, H-2′), 7.91 (br d, J = 8.4 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.3 Hz, 1 H, H-4′), 7.07 (s, 1 H, H-5), 6.87 (t, J = 5.5 Hz, 1 H, NH), 4.40 (t, J = 4.9 Hz, 2 H, OH), 3.45 (dd, J = 5.9, 5.3 Hz, 4 H, CH_2), 3.31 (dd, J = 6.2, 5.9 Hz, 2 H, CH_2), 2.61 (t, J = 6.9 Hz, 2 H, CH_2), 2.55 (t, J = 6.3 Hz, 4 H, CH_2), 1.76 (pentet, J = 6.7 Hz, 2 H, CH_2). Dihydrochloride salt (from EtOH): mp 205−207 °C. Anal. ($C_{20}H_{25}BrN_6O_2\cdot 2HCl$) C, H, N, Cl.

4-[(3-Bromophenyl)amino]-6-[(3-pyridyl)methylamino]-pyrido[3,4-*d*]pyrimidine (5s): from **13** and 3-(aminomethyl)pyridine (37%); ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.78 (s, 1 H, H-8), 8.64 (d, J = 1.7 Hz, 1 H, H-2″), 8.44 (dd, J = 4.7, 1.4 Hz, 1 H, H-4″), 8.41 (s, 1 H, H-2), 8.21 (t, J = 1.9 Hz, 1 H, H-2′), 7.89 (br d, J = 7.9 Hz, 1 H, H-6′), 7.79 (br d, J = 6.2 Hz, 1 H, H-6″), 7.47 (t, J = 6.3 Hz, 1 H, NH), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.35−7.30 (m, 2 H, H-4′,5″), 7.25 (s, 1 H, H-5), 4.60 (d, J = 4.4 Hz, 2 H, CH_2). Dihydrochloride salt (from EtOH): mp 260−262 °C. Anal. ($C_{19}H_{16}BrN_6\cdot 2HCl$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[2-(2-pyridyl)ethyl]amino]pyrido[3,4-*d*]pyrimidine (5t): from **13** and 3-(2-aminoethyl)pyridine (87%); mp (MeOH/H_2O) 170−171.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.53 (dd, J = 4.2, 0.9 Hz, 1 H, H-6″), 8.41 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.90 (br d, J = 7.7 Hz, 1 H, H-6′), 7.73 (td, J =

7.6, 1.7 Hz, 1 H, H-4″), 7.38−7.34 (m, 2 H, H-5′,3″), 7.31 (br d, J = 8.1 Hz, 1 H, H-4′), 7.24 (ddd, J = 8.2, 4.9, 0.8 Hz, 1 H, H-5″), 7.12 (s, 1 H, H-5), 6.88 (t, J = 5.4 Hz, 1 H, NH), 3.65 (dd, J = 7.0, 5.5 Hz, 2 H, CH_2), 3.12 (t, J = 7.2 Hz, 2 H, CH_2). Anal. ($C_{20}H_{17}BrN_6$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[2-(4-imidazolyl)ethyl]amino]pyrido[3,4-*d*]pyrimidine (5u): from **13** and 2-(imidazol-4-yl)ethylamine (66%); mp (MeOH) 200−201 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.87 (br, 1 H, NH), 9.71 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.20 (t, J = 1.8 Hz, 1 H, H-2′), 7.91 (br d, J = 8.0 Hz, 1 H, H-6′), 7.57 (d, J = 0.8 Hz, 1 H, H-2″), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.2 Hz, 1 H, H-4′), 7.13 (s, 1 H, H-5), 6.89 (br s, 1 H, H-5″), 6.85 (t, J = 5.4 Hz, 1 H, NH), 3.51 (dd, J = 7.2, 5.7 Hz, 2 H, CH_2), 2.88 (t, J = 7.3 Hz, 2 H, CH_2). Anal. ($C_{19}H_{18}BrN_7$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[[3-(1-imidazolyl)propyl]amino]pyrido[3,4-*d*]pyrimidine (5v): from **13** and 3-(imidazol-1-yl)propylamine (64%); mp (MeOH/H_2O) 195.5−197 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H, NH), 8.77 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.90 (br d, J = 8.1 Hz, 1 H, H-6′), 7.66 (s, 1 H, H-2″), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.32 (br d, J = 8.2 Hz, 1 H, H-4′), 7.22 (s, 1 H, H-5″), 7.08 (s, 1 H, H-5), 6.98 (t, J = 5.4 Hz, 1 H, NH), 6.91 (s, 1 H, H-4″), 4.11 (t, J = 7.0 Hz, 2 H, CH_2), 3.25 (q, J = 6.1 Hz, 2 H, CH_2), 2.09 (pentet, J = 6.8 Hz, 2 H, CH_2). Anal. ($C_{18}H_{16}BrN_7$) C, H, N.

4-[(3-Bromophenyl)amino]-6-(4-methyl-1-piperazinyl)pyrido[3,4-*d*]pyrimidine (5w): from **13** and 1-methylpiperazine (25%); mp (CH_2Cl_2) 219.5−222 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.75 (s, 1 H, NH), 8.86 (s, 1 H, H-8), 8.48 (s, 1 H, H-2), 8.19 (t, J = 1.7 Hz, 1 H, H-2′), 7.92 (br d, J = 8.1 Hz, 1 H, H-6′), 7.50 (s, 1 H, H-5), 7.39 (t, J = 8.0 Hz, 1 H, H-5′), 7.33 (br d, J = 8.3 Hz, 1 H, H-4′), 3.63 (t, J = 4.8 Hz, 4 H, CH_2), 2.49 (t, J = 4.8 Hz, 4 H, CH_2), 2.26 (s, 3 H, CH_3). Anal. ($C_{18}H_{19}BrN_6$) C, H, N.

4-[(3-Chlorophenyl)amino]-6-(methylamino)pyrido-[3,4-*d*]pyrimidine (7b): from **14** and $MeNH_2$ (82%); mp (MeOH/H_2O) 185.5−187 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.42 (s, 1 H, H-2), 8.11 (t, J = 2.0 Hz, 1 H, H-2′), 7.87 (dd, J = 7.9, 2.0 Hz, 1 H, H-6′), 7.43 (t, J = 8.1 Hz, 1 H, H-5′), 7.18 (dd, J = 7.8, 1.8 Hz, 1 H, H-4′), 7.07 (s, 1 H, H-5), 6.83 (q, J = 4.9 Hz, 1 H, NH), 2.89 (d, J = 4.8 Hz, 3 H, CH_3). Anal. ($C_{14}H_{12}ClN_5$) C, H, N.

6-(Methylamino)-4-[[3-(trifluoromethyl)phenyl]amino]-pyrido[3,4-*d*]pyrimidine (8b): from **15** and $MeNH_2$ (72%); mp (MeOH/H_2O) 172−173 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.85 (s, 1 H, NH), 8.77 (s, 1 H, H-8), 8.42 (s, 1 H, H-2), 8.31 (br s, 1 H, H-2′), 8.27 (br d, J = 8.2 Hz, 1 H, H-6′), 7.64 (t, J = 8.0 Hz, 1 H, H-5′), 7.47 (br d, J = 7.7 Hz, 1 H, H-4′), 7.08 (s, 1 H, H-5), 6.85 (q, J = 4.9 Hz, 1 H, NH), 2.90 (d, J = 5.0 Hz, 3 H, CH_3). Anal. ($C_{15}H_{12}F_3N_5$) C, H, N.

6-(Methylamino)-4-[(3-methylphenyl)amino]pyrido-[3,4-*d*]pyrimidine (9b): from **16** and $MeNH_2$ (74%); mp (MeOH/H_2O) 189−190 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (s, 1 H, NH), 8.72 (s, 1 H, H-8), 8.34 (s, 1 H, H-2), 7.69 (br d, J = 7.9 Hz, 1 H, H-6′), 7.65 (br s, 1 H, H-2′), 7.29 (t, J = 7.8 Hz, 1 H, H-5′), 7.08 (s, 1 H, H-5), 6.96 (br d, J = 7.6 Hz, 1 H, H-4′), 6.76 (q, J = 4.9 Hz, 1 H, NH), 2.88 (d, J = 4.9 Hz, 3 H, CH_3), 2.35 (s, 3 H, CH_3). Anal. ($C_{15}H_{15}N_5$) C, H, N.

6-(Dimethylamino)-4-[(3-methylphenyl)amino]pyrido-[3,4-*d*]pyrimidine (9c): from **16** and dimethylamine (68%); mp 239−241 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.61 (s, 1 H, NH), 8.81 (s, 1 H, H-8), 8.36 (s, 1 H, H-2), 7.69 (br d, J = 7.9 Hz, 1 H, H-6′), 7.64 (br s, 1 H, H-2′), 7.30 (m, 1 H, H-5′), 7.30 (s, 1 H, H-5), 6.98 (br d, J = 7.7 Hz, 1 H, H-4′), 3.17 (s, 6 H, CH_3), 2.35 (s, 3 H, CH_3). Anal. ($C_{16}H_{17}N_5$) C, H, N.

4-[(3-Methylphenyl)amino]-6-[[2-(4-morpholino)ethyl]amino]pyrido[3,4-*d*]pyrimidine (9n): from **16** and 1-(2-aminoethyl)morpholine (78%); mp 168−170 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.56 (s, 1 H, NH), 8.72 (s, 1 H, H-8), 8.34 (s, 1 H, H-2), 7.67 (br d, J = 8.0 Hz, 1 H, H-6′), 7.64 (br s, 1 H, H-2′), 7.29 (t, J = 7.8 Hz, 1 H, H-5′), 7.17 (s, 1 H, H-5), 6.97 (br d, J = 7.5 Hz, 1 H, H-4′), 6.48 (q, J = 5.5 Hz, 1 H, NH), 3.60 (t, J = 4.6 Hz, 4 H, CH_2), 3.31 (m, 2 H, CH_2), 2.60 (t, J = 6.7 Hz, 2 H, CH_2), 2.47 (br m, 4 H, CH_2), 2.35 (s, 3 H, CH_3). Anal. ($C_{20}H_{24}N_6O\cdot0.5\ H_2O$) C, H, N.

4-[(3-Methylphenyl)amino]-6-[[3-(4-morpholino)propyl]amino]pyrido[3,4-*d*]pyrimidine (9o): from **16** and 1-(3-aminopropyl)morpholine (81%); mp 200−203 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (s, 1 H, NH), 8.71 (s, 1 H, H-8), 8.33 (s, 1 H, H-2), 7.67 (br d, J = 8.1 Hz, 1 H, H-6′), 7.63 (br s, 1 H, H-2′), 7.28 (t, J = 7.8 Hz, 1 H, H-5′), 7.10 (s, 1 H, H-5), 6.96 (br d, J = 7.4 Hz, 1 H, H-4′), 6.79 (q, J = 5.6 Hz, 1 H, NH), 3.58 (t, J = 4.6 Hz, 4 H, CH_2), 3.30 (q, J = 6.4 Hz, 2 H, CH_2), 2.42 (t, J = 7.1 Hz, 2 H, CH_2), 2.37 (br m, 4 H, CH_2), 2.35 (s, 3 H, CH_3), 1.80 (pentet, J = 6.9 Hz, 2 H, CH_2). Anal. ($C_{21}H_{26}N_6O\cdot0.5H_2O$) C, H, N.

6-[[2-(4-Imidazolyl)ethyl]amino]-4-[(3-methylphenyl)amino]pyrido[3,4-*d*]pyrimidine (9u): from **16** and 2-(imidazol-4-yl)ethylamine (70%); mp 207−209 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.85 (br, 1 H, NH), 9.58 (s, 1 H, NH), 8.72 (s, 1 H, H-8), 8.34 (s, 1 H, H-2), 7.67 (br d, J = 8.1 Hz, 1 H, H-6′), 7.63 (br s, 1 H, H-2′), 7.56 (br s, 1 H, H-2″), 7.28 (t, J = 7.8 Hz, 1 H, H-5′), 7.15 (s, 1 H, H-5), 6.96 (br d, J = 7.2 Hz, 1 H, H-4′), 6.90 (m, 1 H, NH), 6.78 (t, J = 5.0 Hz, 1 H, H-5″), 3.50 (dd, J = 7.2, 5.7 Hz, 2 H, CH_2), 2.88 (t, J = 6.8 Hz, 2 H, CH_2), 2.34 (s, 3 H, CH_3). Anal. ($C_{19}H_{19}N_7\cdot0.25H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[(2-carboxyethyl)amino]-pyrido[3,4-*d*]pyrimidine (5z), General Example of Method B of Scheme 1. A mixture of **13** (0.20 g, 0.63 mmol) and sodium 2-aminopropanoate (3.4 g, 31 mmol, 50 equiv) (prepared from β-alanine and Na in EtOH) in EtOH (50 mL) was heated at 95 °C for 48 h in a sealed pressure vessel. The solvent was removed under reduced pressure, the residue was dissolved in water and filtered to remove insolubles, and the filtrate was acidified (AcOH) to give **5z** (0.11 g, 45%): mp (MeOH) 276−280 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.25 (br, 1 H, CO_2H), 9.74 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.41 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.90 (br d, J = 8.0 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (br d, J = 8.0 Hz, 1 H, H-4′), 7.14 (s, 1 H, H-5), 6.80 (t, J = 5.5 Hz, 1 H, NH), 3.51 (dd, J = 6.8, 5.7 Hz, 2 H, CH_2), 2.63 (t, J = 7.0 Hz, 2 H, CH_2). Anal. ($C_{16}H_{14}BrN_5O_2$) C, H, N.

The following compounds were prepared similarly.

4-[(3-Bromophenyl)amino]-6-[(carboxymethyl)amino]-pyrido[3,4-*d*]pyrimidine (5x): from **13** and the sodium salt of glycine (34%); mp (MeOH) 234−239 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.54 (br, 1 H, CO_2H), 9.76 (s, 1 H, NH), 8.77 (s, 1 H, H-8), 8.42 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.90 (br d, J = 8.0 Hz, 1 H, H-6′), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.32 (br d, J = 8.2 Hz, 1 H, H-4′), 7.31 (s, 1 H, H-5), 7.01 (t, J = 6.0 Hz, 1 H, NH), 4.05 (d, J = 5.9 Hz, 2 H, CH_2). Anal. ($C_{15}H_{12}BrN_5O_2\cdot0.5H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-6-[*N*-(carboxymethyl)methylamino]pyrido[3,4-*d*]pyrimidine (5y): from **13** and the sodium salt of *N*-methylglycine (79%); mp (MeOH) 225−227 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.59 (br, 1 H, CO_2H), 9.77 (s, 1 H, NH), 8.82 (s, 1 H, H-8), 8.45 (s, 1 H, H-2), 8.19 (t, J = 1.9 Hz, 1 H, H-2′), 7.92 (br d, J = 8.3 Hz, 1 H, H-6′), 7.39 (t, J = 8.0 Hz, 1 H, H-5′), 7.35−7.32 (m, 2 H, H-5,4′), 4.43 (s, 2 H, CH_2), 3.20 (s, 3 H, CH_3). Anal. ($C_{16}H_{14}BrN_5O_2$) C, H, N.

6-Amino-4-[(3-methylphenyl)amino]pyrido[3,4-*d*]pyrimidine (9a). Reaction of **16** (0.254 g, 1 mmol) with 4-methoxybenzylamine (4.1 g, 30 mmol) in DMSO at 100 °C for 2 days gave crude 6-[(4-methoxyphenyl)amino]-4-[(3-methylphenyl)amino]pyrido[3,4-*d*]pyrimidine (0.67 g): ^{1}H NMR [$(CD_3)_2SO$] δ 9.54 (s, 1 H, NH), 8.72 (s, 1 H, H-8), 8.33 (s, 1 H, H-2), 7.66 (br d, J = 8.1 Hz, 1 H, H-6′), 7.62 (br s, 1 H, H-2′), 7.34 (d, J = 8.6 Hz, 2 H, H-2″,6″), 7.29 (t, J = 7.7 Hz, 1 H, H-5′), 7.25 (t, J = 6.4 Hz, 1 H, NH), 7.21 (s, 1 H, H-5), 6.96 (br d, J = 7.6 Hz, 1 H, H-4′), 6.88 (br d, J = 8.6 Hz, 2 H, H-3″,5″), 4.49 (d, J = 6.2 Hz, 2 H, CH_2), 3.71 (s, 3 H, OCH_3), 2.34 (s, 3 H, CH_3).

Hydrolysis of the crude material with refluxing TFA for 1 h and chromatography on alumina, eluting with CH_2Cl_2/MeOH (97:3), gave **9a** (0.14 g, 56%): mp 235.5−237 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.59 (s, 1 H, NH), 8.68 (s, 1 H, H-8), 8.33 (s, 1 H, H-2), 7.67 (br s, 1 H, H-2′), 7.23 (m, 1 H, H-6′), 7.26 (t, J = 8.0

Hz, 1 H, H-5′), 7.18 (s, 1 H, H-5), 6.94 (br d, J = 7.5 Hz, 1 H, H-4′), 6.24 (br s, 1 H, NH), 2.34 (s, 3 H, CH_3). Anal. ($C_{14}H_{13}N_5$) C, H, N.

4-[(3-Bromophenyl)amino]-2-methyl-6-[[2-(4-morpholino)ethyl]amino]pyrido[3,4-*d*]pyrimidine (10n), Scheme 3). A solution of **13** (320 mg, 1 mmol) and nitromethane (0.28 mL, 5 mmol) in DMSO (2 mL) was treated at 25 °C with DBU (0.45 mL, 3 mmol). The mixture was stirred at 25 °C under nitrogen for 24 h and then diluted with 15 mL of 5% aqueous HCl. The resulting suspension was stirred for 20 min, sonicated briefly to break up solids, filtered, and washed well with water and then a small amount of 2-propanol to give 4-[(3-bromophenyl)amino]-6-fluoro-2-methylpyrido[3,4-*d*]pyrimidine hydrochloride **(17)** (307 mg, 84%): mp 230−240 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.30 (br s, exchanges with D_2O, 1 H, NH), 8.78 (s, 1 H, H-8), 8.26 (d, J = 1.7 Hz, 1 H, H-2′), 8.20 (s, 1 H, H-5), 7.91 (dt, J = 2.1, 1.9 Hz, 1 H, H-6′), 7.43−7.36 (m, 2 H, H-4′,5′), 5.14 (br s, exchanges with D_2O, 1 H), 2.85 (s, 3 H, CH_3); ^{19}F NMR δ −74.0; CIMS *m/z* (relative intensity) 333 (MH^+, 100), 335 (78). Anal. ($C_{14}H_{10}N_4BrF$·HCl) C, H, N.

A solution of **17** (207 mg, 0.56 mmol) and 4-(2-aminoethyl)-morpholine (1.1 mL, 8.4 mmol) in DMSO (1.2 mL) was heated at 80 °C for 22 h. The DMSO was removed under reduced pressure, and the residue was dissolved in EtOAc and purified by flash chromatography on silica gel, eluting with EtOAc followed by a gradient (5%, 7.5%, and 10%) of MeOH in EtOAc. The residue from appropriate pooled fractions was dissolved in a minimal volume of hot EtOAc and treated with excess dry HCl in 2-propanol to give **10n** as the dihydrochloride salt (177 mg, 60%): mp >260 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.08 (br s, exchanges D_2O, 1 H), 10.98 (br s, exchanges D_2O, 1 H), 8.61 (s, 1 H, H-8), 8.25 (s, 1 H, H-2′), 7.96 (d, J = 7.7 Hz, 1 H, H-6′), 7.71 (s, 1 H, shifts to 7.49 with D_2O wash, H-5), 7.46−7.22 (m, overlapping with br exchangeable s, 3 H), 4.5 (br s, exchanges D_2O, ca. 1.5 H), 3.99 (d, J = 11 Hz, 2 H), 3.85−3.78 (m, 4 H), 3.55 (d, J = 11.8 Hz, 2 H), 3.36 (d, J = 4.8 Hz, 2 H), 3.18 (q, J = 11 Hz, 2 H), 2.76 (s, 3 H, CH_3); CIMS *m/z* (relative intensity) 443 (MH^+, 58), 445 (53). Anal. ($C_{20}H_{23}N_6OBr$·2HCl) N, H, C: found, 45.9; calcd 46.5.

Measurement 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,[31] and the assays were carried out as reported previously.[8] The substrate used was based on a portion of phospholipase C1, having the sequence Lys-His-Lys-Lys-Leu-Ala-Glu-Gly-Ser-Ala-Tyr472-Glu-Glu-Val. The reaction was allowed to proceed for 10 min at room temperature and then was stopped by the addition of 2 mL of 75 mM phosphoric acid. The solution was then passed through a 2.5-cm phosphocellulose disk which bound the peptide. This filter was washed with 75 mM phosphoric acid (5×), and incorporated label was assessed by scintillation counting in an aqueous fluor. 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 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 anti-phosphotyrosine 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 Ci/mL [^{125}]Iprotein A and then 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, NIH 3T3 fibroblast transfected with the human EGF receptor (EGFR), estrogen-dependent MCF-7 breast, and SK-OV-3 ovarian 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 test compounds on the basis of average cage weight (6 mice/dose group) on the days indicated in the tables. In every experiment, each test compound was evaluated over a range of dose levels ranging from toxic to ineffective. The doses reported in Table 4 are the maximum doses that could be administered without exceeding 10% compound-induced mortality. This dose level, the maximum tolerated dose, allows comparisons to be made among the tested compounds at an equitoxic dose level. The vehicle for **5b** was 6% dimethylacetamide and 94% 50 mM sodium lactate buffer, pH 4.0 (suspension). Compounds **5h**,**k**,**n** and **9b** were administered as isethionate salts in water. Compound dosing solutions 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.[32−35] 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.

Acknowledgment. This work was partially supported by the Auckland Division of the Cancer Society of New Zealand.

References

(1) Bridges, A. J. The epidermal growth factor receptor family of tyrosine kinases and cancer: can an atypical exemplar be a sound therapeutic target? *Curr. Med. Chem.* **1996**, *3*, 167−194.
(2) Fry, D. W. Recent advances in tyrosine kinase inhibitors. *Annu. Rep. Med. Chem.* **1996**, *31*, 151−160.
(3) Rusch, V.; Mendelsohn, J.; Dmitrovsky, E. The epidermal growth factor receptor and its ligands as therapeutic targets in human tumors. *Cytokine Growth Factor Rev.* **1996**, *7*, 133−141.
(4) Yamada, M.; Ikeuchi, T.; Hatanaka, H. The neurotrophic action and signaling of epidermal growth factor. *Prog. Neurobiol.* **1997**, *51*, 19−37.
(5) Lupu, R.; Lippmann, M. E. The role of *erbB*2 signal transduction pathways in human breast cancer. *Breast Cancer Res. Treat.* **1993**, *27*, 83−93.
(6) Hickey, K.; Grehan, D.; Reid, I. M.; O'Briain, S.; Walsh, T. N.; Hennessy, T. P. J. Expression of epidermal growth factor receptor and proliferating cell nuclear antigen predicts response of esophageal squamous cell carcinoma to chemoradiotherapy. *Cancer* **1994**, *74*, 1693−1698.

(7) Delarue, J. C.; Terrier, P.; Terrier-Lacombe, M. J.; Mouriesse, H.; Gotteland, M.; May-Levin, F. Combined overexpression of c-*erbB*-2 protein and epidermal growth factor receptor (EGF.-R) could be predictive of early and long-term outcome in human breast cancer: a pilot study. *Bull. Cancer* **1994**, *81*, 1067−1077.
(8) Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science* **1994**, *265*, 1093−1095.
(9) Ward, W. H. J.; Cook, P. N.; Slater, A. M.; Davies, D. H.; Holdgate, G. A.; Green, L. R. Epidermal growth factor receptor tyrosine kinase. Investigation of catalytic mechanism, structure-based searching and discovery of a potent inhibitor. *Biochem. Pharmacol.* **1994**, *48*, 659−666.
(10) Rewcastle, G. W.; Denny, W. A.; Bridges, A. J.; Zhou, H.; Cody, D. R.; McMichael, A.; Fry, D. W. Tyrosine kinase inhibitors. 5. Synthesis and structure−activity relationships for 4-[(phenylmethyl)amino]- and 4-(phenylamino)quinazolines as potent adenosine 5′-triphosphate binding site inhibitors of the tyrosine kinase domain of the epidermal growth factor receptor. *J. Med. Chem.* **1995**, *38*, 3482−3487.
(11) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D. H.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine kinase inhibitors. 8. An unusually steep structure−activity relationship for analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor. *J. Med. Chem.* **1996**, *39*, 267−276.
(12) Wakeling, A. E.; Barker, A. J.; Davies, D. H.; Brown, D. S.; Green, L. R.; Cartlidge, S. A.; Woodburn, J. R. Specific inhibition of epidermal growth factor receptor tyrosine kinase by 4-anilinoquinazolines. *Breast Cancer Res. Treat.* **1996**, *38*, 67−73.
(13) Woodburn, A. J.; Barker, A. J.; Gibson, K. H.; Ashton, S. E.; Wakeling, A. E.; Curry, B. J.; Scarlett, L.; Henthorn, L. R. ZD 1839, an epidermal growth factor tyrosine kinase inhibitor selected for clinical trial development. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 633 (Abstract 4251).
(14) Iwata, K.; Miller, P. E.; Barbacci, E. G.; Arnold, L.; Doty, J.; DiOrio, C. I.; Pustilnik, L. R.; Reynolds, M.; Theleman, A.; Sloan, D.; Moyer, J. D. CP-358,774: a selective EGFR kinase inhibitor with potent antiproliferative activity against HN5 head and neck tumor cells. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 633 (Abstract 4248).
(15) Rewcastle, G. W.; Palmer, B. D.; Thompson, A. M.; Bridges, A. J.; Cody, D. R.; Zhou, H.; Fry, D. W.; McMichael, A.; Denny, W. A. Tyrosine kinase inhibitors. 10. Isomeric 4-[(3-bromophenyl)-amino]pyrido[*d*]pyrimidines are potent ATP binding site inhibitors of the tyrosine kinase function of the epidermal growth factor receptor. *J. Med. Chem.* **1996**, *39*, 1823−1835.
(16) Fry, D. W.; Nelson, J. M.; Slintak, V.; Keller, P. R.; Rewcastle, G. W.; Denny, W. A.; Zhou, H.; Bridges, A. J. Biochemical and antiproliferative properties of 4-[ar(alk)ylamino]pyridopyrimidines, a new chemical class of potent and specific epidermal growth factor receptor tyrosine kinase inhibitor. *Biochem. Pharmacol.* **1997**, *54*, 877−887.
(17) Plowman, G. D.; Green, J. M.; Culouscou, J.-M.; Carlton, G. W.; Rothwell, V. M.; Buckley, S. Heregulin induces tyrosine phosphorylation of HER4/P180^{erbB4}. *Nature* **1993**, *366*, 473−475.
(18) Sliwkowski, M. X.; Schaefer, G.; Akita, R. W.; Lofgren, J. A.; Fitzpatrick, V. D.; Nuijens, A.; Fendly, B. M.; Cerione, R. A.; Vandlen, R. L.; Carraway, K. L. Coexpression of *erbB*2 and *erbB*3 reconstitutes a high-affinity receptor for heregulin. *J. Biol. Chem.* **1994**, *269*, 14661−14665.
(19) Palmer, B. D.; Trumpp-Kallmeyer, S.; Fry, D. W.; Nelson, J. M.; Showalter, H. D. H.; Denny, W. A. Tyrosine Kinase Inhibitors. 11. Soluble analogues of pyrrolo- and pyrazoloquinazolines as epidermal growth factor receptor inhibitors: synthesis, biological evaluation, and modeling of the mode of binding. *J. Med. Chem.* **1997**, *40*, 1519−1529.
(20) Thompson, A. M.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Nelson, J. A.; Showalter, H. D. H.; Roberts, B. J.; Vincent, P. W.; Denny, W. A. 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. *J. Med. Chem.* **1997**, *40*, 3915−3925.
(21) Rewcastle, G. W.; Denny, W. A.; Winters, R. T.; Colbry, N. L.; Showalter, H. D. H. Synthesis of 6-substituted pyrido[3,4-*d*]-pyrimidin-4(3*H*)-ones via directed lithiation of 2-substituted 5-aminopyridine derivatives. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2221−2226.
(22) Kohn, K. W.; Orr, A.; O′Connor, P. M. Synthesis and DNA-sequence selectivity of a series of mono- and difunctional 9-aminoacridine nitrogen mustards. *J. Med. Chem.* **1994**, *37*, 67−72.
(23) Thompson, A. M.; Bridges, A. J.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine kinase inhibitors. 7. 7-Amino-4-(phenylamino)- and 7-amino-4-[(phenylmethyl)amino]pyrido[4,3-*d*]pyrimidines; a new class of inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor. *J. Med. Chem.* **1995**, *38*, 3780−3788.
(24) Traxler, P. M.; Furet, P.; Mett, H.; Buchdunger, E.; Meyer, T.; Lydon, N. 4-(Phenylamino)pyrrolopyrimidines: potent and selective ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase. *J. Med. Chem.* **1996**, *39*, 2285−2292.
(25) **(**25) Rewcastle, G. W.; Bridges, A. J.; Fry, D. W.; Rubin, J. R.; Denny, W. A. Tyrosine kinase inhibitors. 12. Synthesis and structure−activity relationships for 6-substituted 4-(phenylamino)pyrimido[5,4-*d*]pyrimidines designed as inhibitors of the epidermal growth factor receptor. *J. Med. Chem.* **1997**, *40*, 1820−1826.
(26) Jones, J. A.; Howard, C. T.; Roberts, B. J.; Elliott, W. L. In vivo establishment and characterization of a murine fibroblast cell line transfected with the human EGF receptor. *Proc. Am. Assoc. Cancer Res.* **1994**, *35*, 84 (Abstract 499).
(27) Böhme, H.; Osmers, K. d-Halo amines. XLII. Reactions in the imidazolidine and oxazolidine series. *Chem. Ber.* **1972**, *105*, 2237−2245.
(28) Tkaczynski, T. Synthesis of ethylene amines. VIII. Condensation of *N*-alkyl-*N*′-acetylethylenediamines with ethylene oxide and cyclization of the reaction products to *N*-alkylpiperazines. *Acta Polon. Pharm.* **1966**, *23*, 15−18; *Chem. Abstr.* **1966**, *64*, 17594*d*.
(29) Preston, R. K.; Peck, R. M.; Breuninger, E. R.; Miller, A. J.; Creech, H. J. Further investigations of heterocyclic alkylating agents**.** *J. Med. Chem.* **1964**, ***7***, 471−480.
(30) Gilman, H.; Sherman, C. S.; Price, C. C.; Elderfield, R. C.; Maynard, J. T.; Reitsema, R. H.; Tolman, L.; Massie, S. P.; Marshall, F. J.; Goldman, L. Synthesis of 1-diethylamino-2,3-epoxypropane, 3-diethylamino-2-hydroxypropylamine, and 4-diethylamino-3-hydroxybutylamine**.** *J. Am. Chem. Soc.* **1946**, *68*, 1291−1293.
(31) Gill, G. N.; Weber, W. Purification of functionally active epidermal growth factor receptor protein using a competitive antagonist monoclonal antibody and competitive elution with epidermal growth factor. *Methods Enzymol.* **1987**, *146*, 82−88.
(32) Schabel, F. M., Jr.; Griswold, D. P., Jr.; Laster, W. R., Jr.; Corbett, T. H.; Lloyd, H. H. Quantitative evaluation of anticancer agent activity in experimental animals. *Pharmacol. Ther.* **1977**, *1*, 411−435.
(33) Leopold, W. R.; Nelson, J. M.; Plowman, J.; Jackson, R. C. Anthrapyrazoles, a new class of intercalating agents with high-level, broad spectrum activity against murine tumors. *Cancer Res.* **1985**, *45*, 5532−5539.
(34) Elliott, W. L.; Howard, C. T.; Dykes, D. J.; Leopold, W. R. Sequence and schedule-dependent synergy of trimetrexate in combination with 5-fluorouracil in vitro and in mice. *Cancer Res.* **1989**, *49*, 5586−5590.
(35) Elliott, W. L.; Roberts, B. J.; Howard, C. T.; Leopold, W. R. Chemotherapy with [SP-4-3-(*R*)]-[1,1-cyclobutanedicarboxylato-(2-)](2-methyl-1,4-butanediamine-N,N′) platinum (CI-973,NK121) in combination with standard agents against murine tumors in vivo. *Cancer Res.* **1994**, *54*, 4412−4418.
(36) Fry, D. W.; Kraker, A. J.; Connors, R. C.; Elliott, W. L.; Nelson, J. M.; Showalter, H. D. H.; Leopold, W. R. Strategies for the discovery of novel tyrosine kinase inhibitors with anticancer activity. *Anti-Cancer Drug Des.* **1994**, *9*, 331−351.

JM970641D