Tyrosine Kinase Inhibitors. 18. 6-Substituted 4-Anilinoquinazolines and 4-Anilinopyrido[3,4-*d*]pyrimidines as Soluble, Irreversible Inhibitors of the Epidermal Growth Factor Receptor

Jeff B. Smaill,[†] H. D. Hollis Showalter,[#] Hairong Zhou,[#] Alexander J. Bridges,[#] Dennis J. McNamara,[#] David W. Fry,[#] James M. Nelson,[#] Veronika Sherwood,[#] Patrick W. Vincent,[#] Bill J. Roberts,[#] William L. Elliott,[#] and William A. Denny*[,†]

Auckland Cancer Society Research Centre, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92019, Auckland, New Zealand, and Pfizer Global Research and Development, Ann Arbor Laboratories, 2800 Plymouth Road, Ann Arbor, Michigan 48106-1047

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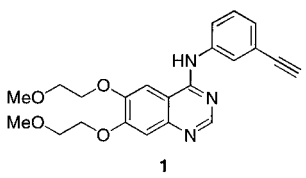
4-Anilinoquinazoline- and 4-anilinopyrido[3,4-*d*]pyrimidine-6-acrylamides are potent pan-*erb*B tyrosine kinase inactivators, and one example (CI-1033) is in clinical trial. A series of analogues with a variety of Michael acceptor units at the 6-position were prepared to define the structural requirements for irreversible inhibition. A particular goal was to determine whether additional functions to increase solubility could be appended to the Michael acceptor. Substituted acrylamides were prepared by direct acylation of the corresponding 6-amines with the requisite acid or acid chloride. Vinylsulfonamide derivatives were obtained by acylation of the amines with chloroethylsulfonyl chloride followed by base-promoted elimination. Vinylsulfone and vinylsulfine derivatives were prepared by oxidation and base elimination of a hydroxyethylthio intermediate. The compounds were evaluated for their inhibition of phosphorylation of the isolated EGFR enzyme and for inhibition of EGF-stimulated autophosphorylation of EGFR in A431 cells and of heregulin-stimulated autophosphorylation of *erb*B2 in MDA-MB 453 cells. Substitution at the nitrogen of the acrylamide was tolerated only with a methyl group; larger substituents were dystherapeutic, and no substitution at all was tolerated at the acrylamide α-carbon. In contrast, while electron-donating groups at the acrylamide *β*-carbon were not useful, even quite large electron-withdrawing groups (which increase its electrophilicity) were tolerated. A series of derivatives with solubility-enhancing substituents linked to the acrylamide *β*-carbon via amides were potent irreversible inhibitors of isolated EGFR (IC_{50}s = 0.4−1.1 nM), with weakly basic morpholine and imidazole derivatives being the best. Vinylsulfonamides were also potent and irreversible inhibitors, but vinylsulfones and vinylsulfines were reversible and only poorly active. Two compounds were evaluated against A431, H125, and MCF-7 xenografts in nude mice but were inferior in these assays to the clinical trial compound CI-1033.

Introduction

Overexpression of the epidermal growth factor receptor (EGFR) has been reported in a significant number of human tumors and is associated with poor prognosis.[1,2] Inhibition of growth signal pathways mediated through EGFR tyrosine autophosphorylation is thus of therapeutic interest, and inhibitors of this process have been widely sought as potential anticancer drugs.[3−5] 4-Anilinoquinazolines and related 4-anilinopyrido[*d*]-pyrimidines have been shown to be potent and selective reversible inhibitors of both isolated EGFR and EGF-stimulated EGFR autophosphorylation in cells, via competitive binding to the ATP site,[6,7] and two compounds of this type, CP-358,774 (**1**) and ZD 1839 (Iressa) (**2**), are in clinical trial.[8,9]

In expectation that the high levels of intracellular ATP in some cell lines may make it difficult to achieve sufficiently high intracellular levels of such inhibitors



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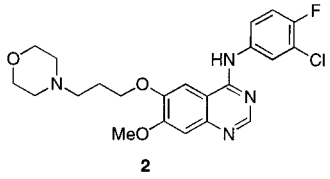

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to shut-down EGF-stimulated autophosphorylation for long periods, we[10,11] and others[12] have been exploring the use of irreversible inhibitors. We have recently reported[13−15] that 6- and (to a lesser extent) 7-acrylamide analogues of the 4-anilinoquinazolines and pyrido[*d*]pyrimidines (e.g., **3** and **4**) act at the ATP binding domain of EGFR, specifically alkylating an adjacent Cys-773 residue and irreversibly shutting down kinase activity. The 6-acrylamides are irreversible inhibitors of both EGFR and *erb*B2 autophosphorylation and show significantly improved in vivo antitumor activity compared to closely related reversible analogues.[13] They tolerate a wide range of structural variations in the molecule with retention of irreversibility and potency, including substitution at the vacant 7-position of the quinazoline nucleus with a range of amine-bearing side

* To whom correspondence should be addressed. Tel: 64 9 3737 599, ext. 6144. Fax: 64 9 3737 502. E-mail: b.denny@auckland.ac.nz.
[†] The University of Auckland.
[#] Pfizer Global Research and Development.

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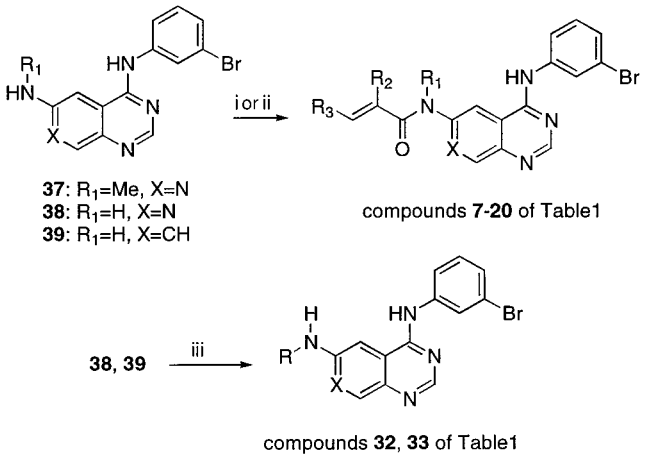
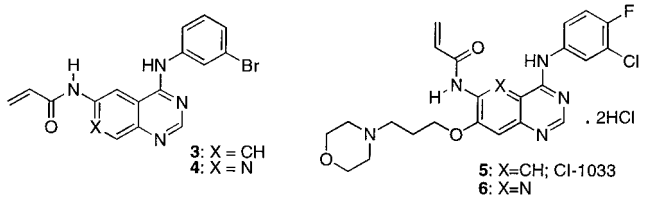
Scheme 1[a]



[a] (i) $R_3CH{=}C(R_2)CO_2H$/EDCl·HCl/pyridine/THF/DMA; (ii) $R_3CH{=}C(R_2)COCl$/DMAP (cat.)/THF/Et_3N; (iii) $ClCH_2CH_2SO_2Cl$/DMAP (cat.)/THF/Et_3N.

chains, and provide a class of soluble, orally active, potent, selective, and irreversible inhibitors of the EGFR family of tyrosine kinases.[15] This novel class of compounds is exemplified by CI-1033 (**5**) which has recently begun phase I clinical trials.[15,16]



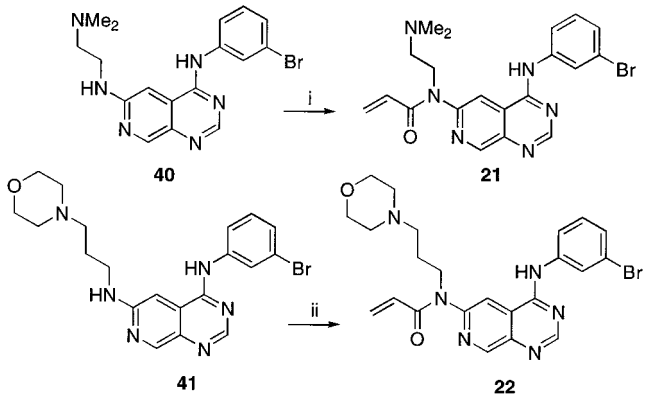
Attempts to develop a soluble pyrido[*d*]pyrimidine analogue of **5**, by introducing amine-bearing soluble side chains at the 7-position of a pyrido[3,2-*d*]pyrimidine nucleus, met with only limited success.[15] While these compounds (e.g., **6**) showed excellent potency for inhibition of isolated EGFR enzyme, they were considerably less potent in cellular assays when compared to the analogous quinazolines. This lack of potency has in part been attributed to the increased reactivity of the acrylamide moiety toward cellular glutathione.[15]

In further development of this class, we now report the synthesis and biological activity of a range of 4-anilinoquinazolines and pyrido[3,4-*d*]pyrimidines substituted at the 6-position with a variety of Michael acceptors apart from the parent acrylamide. This work sought to define the structural requirements of the Michael acceptor necessary to provide irreversible inhibition of EGFR, with the aim of developing one that can tolerate substitution with a solubility-enhancing side chain while retaining irreversible inhibition, potency, and selectivity for EGFR.

Chemistry

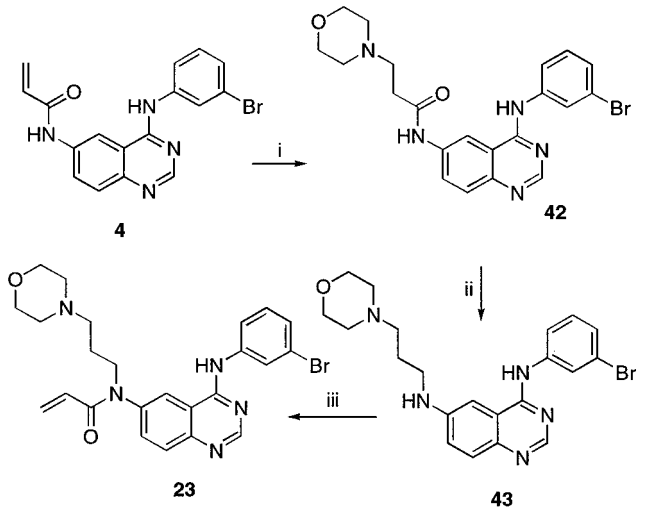
The substituted acrylamides **7**−**20** of Table 1 were obtained by direct acylation of the known 6-amino derivatives **37**−**39**, with either the requisite acid under EDCI·HCl-promoted coupling or with the acid chloride in the presence of catalytic DMAP and a base such as triethylamine or pyridine. The vinylsulfonamide derivatives **32** and **33** of Table 1 were similarly obtained by acylation of the amines **38** and **39** with chloroethylsulfonyl chloride, followed by base-promoted in situ elimination of HCl, to generate the vinyl moiety (Scheme 1). The pyrido[3,4-*d*]pyrimidines **21** and **22** of Table 1 were obtained by acylation of the known amines **40** and **41** with acrylic acid (EDCI·HCl-promoted) and acryloyl chloride, respectively (Scheme 2).

Scheme 2[a]



[a] (i) $CH_2{=}CHCO_2H$/EDCl·HCl/pyridine; (ii) $CH_2{=}CHCOCl$/DMAP (cat.)/Et_3N.

Scheme 3[a]

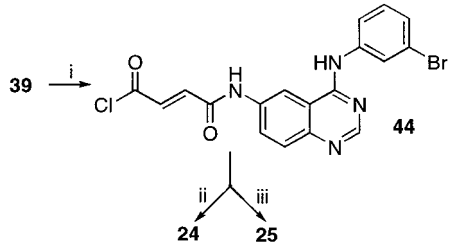


[a] (i) Morpholine/*p*-TsOH/THF; (ii) BH_3·DMS/THF; (iii) $CH_2{=}CHCO_2H$/EDCl·HCl/Et_3N/DMF.

Synthesis of the quinazoline **23** required first the synthesis of amine **43**. This was obtained by the acid-catalyzed Michael addition of morpholine to acrylamide **4**, followed by borane−dimethyl sulfide reduction of the amide functionality. Amine **43** was then coupled with acrylic acid using EDCI·HCl to give acrylamide **23** of Table 1 (Scheme 3). Compounds **24** and **25** of Table 1, bearing an acrylamide moiety substituted in the 3-position with a solubility-enhancing ester and amide functionality, respectively, were obtained from the reaction of 3-(*N*,*N*-dimethylamino)propan-1-ol and *N*,*N*-dimethyl-1,3-propanediamine. The key intermediate was acid chloride **44**, in turn prepared from acylation of amine **39** with fumaryl chloride (Scheme 4).

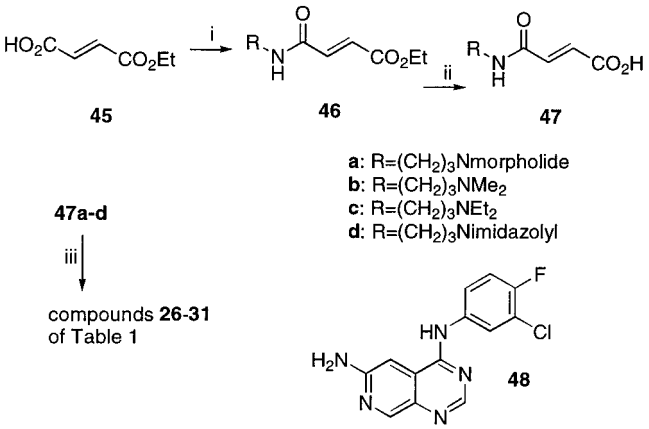
EDCI·HCl-promoted coupling of acids **47a**−**d** with the amines **37**, **38**, and **48** provided compounds **26**−**31** of Table 1. The acids **47a**−**d** needed for this work were synthesized from (*E*)-but-2-enedioic acid monoethyl ester (**45**). Conversion to the acid chloride with oxalyl chloride and then condensation with the required amine

Scheme 4[a]



[a] (i) ClOCCH=CHCOCl/THF; (ii) $Me_2N(CH_2)_3OH$/THF; (iii) $Me_2N(CH_2)_3NH_2$/THF.

Scheme 5[a]



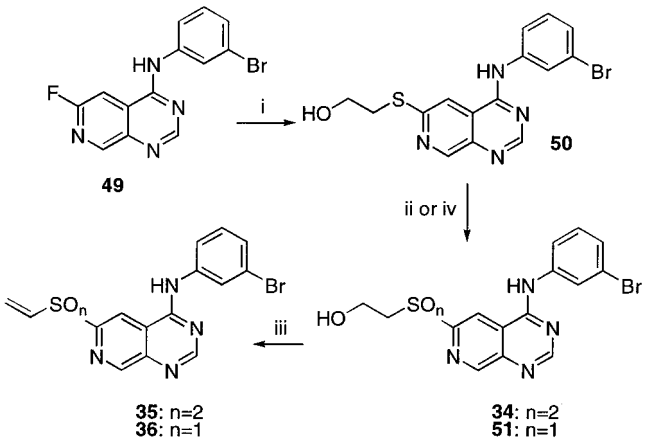
[a] (i) Oxalyl chloride/THF/20 °C/1 h, then RNH_2; (ii) aq Et_3N/60 °C/45 min or LiOH/aq MeOH/20 °C/2 h; (iii) EDCl·HCl/pyridine/0 °C/1 h and **37**, **38**, or **48**.

side chains gave the amide esters **46a**−**d**, which were subsequently converted to the acids **47a**−**d** by alkaline hydrolysis (Scheme 5). Synthesis of the vinylsulfone **35** and vinylsulfine **36** of Table 1 was achieved by base-catalyzed elimination of the respective mesylates of the hydroxyethyl compounds **34** and **51**. These were in turn prepared from the reaction of 2-mercaptoethanol with 6-fluoropyrido[3,4-*d*]pyrimidine (**49**) to give the hydroxyethyl sulfide **50**, which was oxidized with either MCPBA to give **34** or Davis' reagent to give **51** (Scheme 6).

Results and Discussion

Alternative Michael Acceptors. Table 1 lists the structures and physicochemical properties of a series of 4-(3-bromoanilino)quinazolines and pyrido[3,4-*d*]pyrimidines substituted at the 6-position with a variety of Michael acceptors. We have previously shown[14] that quinazoline- and pyrido[3,4-*d*]pyrimidine-6-acrylamides (e.g., **3** and **4**) show broadly similar results in these assays, and the anilino ring was kept constant in order to facilitate intercomparisons of the different Michael acceptors. Potencies ($IC_{50[app]}$ in nM) were determined for both inhibition of phosphorylation of a random glutamic acid/tyrosine copolymer substrate by isolated EGFR enzyme and inhibition of EGF-stimulated autophosphorylation of EFGR in A431 cells. The type of inhibition of the isolated EGFR enzyme is also listed. Irreversible inhibition is defined[13−15] as 80% or greater inhibition after a 10-min exposure to drug, followed by drug washout and re-stimulation by EGF 8 h later. Drugs that produced 20−80% inhibition were designated as partially irreversible (although in reality they

Scheme 6[a]



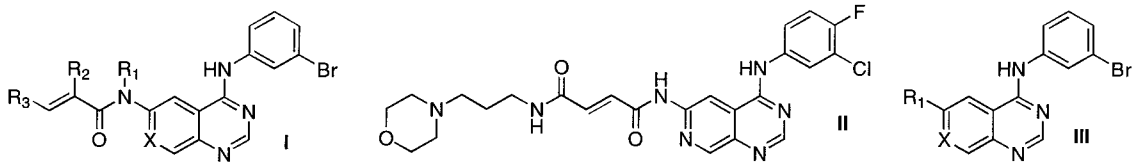
[a] (i) $HSCH_2CH_2OH/Cs_2CO_3$/DMSO/50 °C/2 h; (ii) MCPBA/$CHCl_3$/20 °C/4 h; (iii) MsCl/Et_3N/CH_2Cl_2/0−5 °C/2.5 h; (iv) Davis' reagent/$CHCl_3$.

can almost certainly fully inactivate the enzyme via alkylation given enough time). Those that produced less than 20% inhibition were classified as reversible. For compounds capable of rapid and complete alkylation of the enzyme, the IC_{50} values derive essentially from titrating the enzyme activity in a stoichiometric manner and for this reason are designated as apparent IC_{50}s ($IC_{50[app]}$).[14,15] The concentration of EGFR in the isolated enzyme assays is calculated at 1.18 nM and was held as constant as possible (<10% variation). The $IC_{50[app]}$ values are an average of at least two separate determinations.

The present study investigated several different types of Michael acceptors in addition to the original acrylamides **3** and **4**. Compounds **7**−**20** represent 11 modifications of the parent acrylamide itself, using both quinazoline and pyrido[3,4-*d*]pyrimidine chromophores, seeking acceptable positions for substitution that would allow attachment of a solubility-enhancing function. The *N*-methyl analogue **7** showed irreversible inhibition of the isolated enzyme with high potency ($IC_{50[app]}$ = 0.17 nM compared with 0.91 nM for **3**). Although there was a small loss of potency in the cellular assay, this position appeared suitable for further substitution (but see later). In contrast, the α-methylacrylamides **8** and **9** showed lower potencies in both the enzyme and cellular assays compared to **3** and **4** and a complete loss of irreversibility, indicating that substitution at this position of the Michael acceptor is not tolerated.

A larger number of different substitutions were investigated at the acrylamide *β*-position (compounds **10**−**20**). Compounds **10** and **11** show that a *β*-methyl substituent gives a small (2−3-fold) reduction in potency in the autophosphorylation assay, but more importantly resulted in a reduction in the rate of enzyme alkylation, providing only partially irreversible inhibition of EGFR. This is consistent with introduction of a small amount of steric bulk and electron donation to the Michael acceptor double bond, reducing its electrophilicity and therefore its alkylating ability. This is supported by the data for the *cis*-chloro and trifluoromethyl analogues **12** and **13**. These groups have similar steric properties to the methyl groups in **10** and **11**, but these more electron-withdrawing groups increase the electrophilicity of the double bond of the Michael acceptor, resulting in fully

Table 1. EGFR Inhibitory Properties of 6-Substituted 4-Anilinoquinazolines and 4-Anilinopyrido[3,4-*d*]pyrimidines Bearing Various Michael Acceptors



no.	family	X	R_1	R_2	R_3	$IC_{50[app]}$ (nM)[a] EGFR	IC_{50} (nM)[b] A431	irrev inhib[c]
3[d]	I	N	H	H	H	0.91	3.4	yes
4[d]	I	C	H	H	H	0.70	2.7	yes
5[e]						1.5	7.4	yes
7	I	N	Me	H	H	0.17	13	yes
8	I	N	H	Me	H	1.6	44	no
9	I	C	H	Me	H	1.2	16	no
10	I	N	H	H	Me	0.50	7.7	partially
11	I	C	H	H	Me	0.55	8.7	partially
12	I	N	H	H	*cis*-Cl	0.69	20	yes
13	I	C	H	H	CF_3	1.75	35	yes
14	I	N	H	H	$CH{=}CH_2$	1.1	27	partially
15	I	C	H	H	$={CH_2}$	1.6	120	partially
16	I	N	H	H	Ph	9.1	77	partially
17	I	C	H	H	COMe	1.2	1039	partially
18	I	C	H	H	COOH	0.37	>500	yes
19	I	C	H	H	COOEt	2.7	64	partially
20	I	N	H	H	COOEt	1.5	51	yes
21	I	N	$(CH_2)_2NMe_2$	H	H	4.2	2282	partially
22	I	N	$(CH_2)_3$-*N*-morpholinyl	H	H	2.7	156	no
23	I	C	$(CH_2)_3$-*N*-morpholinyl	H	H	3.3	194	no
24	I	C	H	H	$COO(CH_2)_3NMe_2$	2.4	108	yes
25	I	C	H	H	$CONH(CH_2)_3NMe_2$	0.44	59	yes
26	I	N	H	H	$CONH(CH_2)_3NMe_2$	1.1	57	yes
27	I	N	H	H	$CONH(CH_2)_3NEt_2$	0.73	21	yes
28	I	N	H	H	$CONH(CH_2)_3$-*N*-morpholinyl	0.81	8.8	yes
29	I	N	H	H	$CONH(CH_2)_3$-*N*-imidazolyl	0.56	14	yes
30	I	N	Me	H	$CONH(CH_2)_3NMe_2$	1.45	193	partially
31	II					0.61	14	yes
32	III	N	$NHSO_2CH{=}CH_2$			0.76	2.4	yes
33	III	C	$NHSO_2CH{=}CH_2$			1.4	2.7	yes
34	III	N	$SO_2CH_2CH_2OH$			93.5		no
35	III	N	$SO_2CH{=}CH_2$			0.43	>500	yes
36	III	N	$SOCH{=}CH_2$			4.6	340	no

[a] Concentration to inhibit by 50% the phosphorylation of a polyglutamic acid/tyrosine random copolymer by EGFR enzyme (prepared from human A431 carcinoma cell vesicles by immunoaffinity chromatography). Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [b] Concentration to inhibit by 50% the autophosphorylation of EGFR in A431 cells (detected by immunoblotting). [c] Irreversible inhibition is defined as >80% inhibition of formation of phosphorylated EGFR in A431 cells 8 h after washing cells free of the inhibitor. [d] Data from ref 14. [e] Data from ref 15.

irreversible compounds. We have previously reported[15] that increased reactivity of the Michael acceptor can result in increased background alkylation of cellular thiols, such as glutathione, and therefore a reduction in cellular potency for inhibition of EGFR. It is likely therefore that a balance is required, between increased reactivity of the Michael acceptor and increased steric bulk, to provide compounds that are still capable of rapid alkylation of the target Cys-773 without displaying significantly increased background alkylation. The irreversibility assay in conjunction with the cellular assay for inhibition of EGFR autophosphorylation provides a measure of the success of this balancing act.

Substitution at the β-position with an unsaturated double bond, an allene functionality, or a phenyl group (compounds **14**−**16**, respectively) resulted in only partially irreversible inhibition, suggesting the mild electron-withdrawing abilities of these groups was not sufficient to overcome their respective steric hindrances. Compounds possessing more strongly electron-withdrawing β-carbonyl substituents such as methyl ketone (**17**), acid (**18**), and ethyl ester (**19** and **20**) groups at the 3-position showed more promise, with **18** and **20** possessing potent activity against the isolated enzyme ($IC_{50[app]} = 0.37$ and 1.5 nM, respectively) while also being fully irreversible. However, acid **18** showed a large loss of potency in the cellular assay ($IC_{50} > 500$ nM), presumably due to its lack of ability to permeate the cell membrane. Ester **20** also showed a 15-fold loss of potency when compared to the parent acrylamide **3** in the cellular assay, possible due to partial ester hydrolysis to the nonpotent acid.

Attachment of Soluble Side Chains at the Acrylamide Nitrogen. The *N*-methyl analogue **7** retained potent irreversible inhibitory activity against the EGFR enzyme. However, substitution of the acrylamide nitrogen with the larger *N*,*N*-dimethylaminoethyl or morpholinopropyl groups (compounds **21**−**23**, respectively) resulted in a much larger attenuation of potency (46−671-fold), with an associated loss of irreversible inhibition of cellular autophosphorylation. This suggests that there is only minimal steric tolerance (no bigger than a methyl group) for substitution at the acrylamide nitrogen.

Attachment of Soluble Side Chains at the Acrylamide β-Carbon. The tolerance for carbonyl substituents at the acrylamide β-position led to the synthesis of a small subset of such carbonyl-linked soluble side chain derivatives (**24**−**31**). The ester-linked quinazoline

Table 2. Comparative Inhibition of Autophosphorylation of EGFR and *erb*B2 by Selected Analogues

	IC_{50} (nM)			IC_{50} (nM)	
no.	EGFR[a]	*erb*B2[b]	no.	EGFR[a]	*erb*B2[b]
5[c]	7.4	9.0	**27**	21	14
24	108	110	**28**	8.8	5.0
25	59	207	**29**	14	8.1
26	57	13	**31**	14	24

[a] Concentration to inhibit by 50% the EGF-stimulated autophosphorylation of EGFR in A431 cells. Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [b] Concentration to inhibit by 50% the heregulin-stimulated autophosphorylation of *erb*B2 in MDA-MB 453 cells. Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [c] Data from ref 15.

24 was fully irreversible but not particularly potent in the cellular assay (IC_{50} = 108 nM). This loss of potency may be due to partial ester hydrolysis to the (nonpotent) parent acid **18**. The more stable amide derivatives (**25**−**29**, **31**), containing a variety of cationic side chains, were all fully irreversible, displayed good potency against isolated EGFR ($IC_{50[app]}$ = 0.4−1.1 nM), and were more potent than the ester analogue **24**. The most potent of these were the weakly basic morpholine and imidazole derivatives **28** and **29** (IC_{50}s for inhibition of autophosphorylation = 8.8 and 14 nM, respectively). A comparison of the 3-bromo- and 3-chloro-4-fluoroanilino (the anilino substituents employed in **5**), in analogues **28** and **31**, showed that the 3-bromo side chain gives a slight advantage in outright potency in the cellular assay (IC_{50} = 8.8 compared to 14 nM). Interestingly, the soluble *N*-methyl analogue **30** showed a large loss of potency (IC_{50} = 193 nM) and a partial loss of irreversibility in the cellular assay compared to the *N*-methyl compound **7** (which was fully irreversible). Presumably the introduction of the methyl group at the acrylamide nitrogen, in combination with the bulky soluble side chain at the acrylamide 3-position, is too much of a steric impediment, preventing the positioning of the Michael acceptor in a conformation where it can alkylate the enzyme effectively. The solubility-enhanced analogues showing irreversible inhibition (**24**−**29**, **31**) were compared for their ability to inhibit both EGF-stimulated autophosphorylation of EGFR in A431 cells and heregulin-stimulated autophosphorylation of *erb*B2 in MDA-MB 453 cells (Table 2). With the exception of **25**, the compounds were equipotent inhibitors of both receptors, with IC_{50} values comparable to those of **5**. Such broad-spectrum inhibition of different *erb*B family members is a potential advantage, since signaling through this pathway occurs via both homo- and heterodimers.

Comparison of Quinazoline and Pyrido[3,4-*d*]-pyrimidine Chromophores. Several pairs of quinazolines and pyrido[3,4-*d*]pyrimidines (**8/9**, **10/11**, **19/20**, **22/23**, and **32/33**) were evaluated to determine the influence of the 7-aza atom. We have previously[14] shown little activity difference between these chromophore classes, and the present results confirm this (Table 1).

Non-Acrylamide Michael Acceptors. The vinylsulfonamide was studied as an alternate Michael acceptor that would allow subsequent substitution with soluble cationic side chains. Compounds **32** and **33** were both able to irreversibly inhibit EGFR autophosphorylation and were very potent in the cellular assay (IC_{50}s = 2.4 and 2.7 nM, respectively). However, subsequent in vivo experiments with vinylsulfonamide **32** suggested that it was unstable in biological systems, and this avenue was therefore not pursued. The vinylsulfone **35** was an irreversible inhibitor but showed very poor potency in the cellular assay (IC_{50} > 500 nM), while its hydroxyethyl precursor **34** and the vinylsulfine **36** were poorly active and were reversible inhibitors.

Table 3. In Vivo Antitumor Properties of Selected 4-Anilinoquinazolines and 4-Anilinopyrido[3,4-*d*]pyrimidine-6-acrylamides

no.	tumor	dose (mg/kg)	schedule[a]	wt change (g)[b]	T/C (%) last therapy day[c]	T−C[d] (days)
5[e]	A431	18[f]	days 10−24	−1.0	0	41.3
7	A431	200 HDT[g]	days 12−26	−0.1	62	5.2
	H125	200 HDT	days 15−29	−0.4	56	7.3
	MCF-7	200 HDT	days 11−25	−0.3	88	1.5
31	A431	200 HDT	days 12−26	−0.1	70	5.2
	H125	200 HDT	days 15−29	−0.3	23	7.9
	MCF-7	200 HDT	days 17−31	+	124	6.8

[a] Compound **5** was administered as a solution of the isethionate salt in 50 mM sodium lactate buffer, pH 4.0. Compounds **7** and **31** were administered as suspensions in 0.5% methylcellulose in water. All three compounds were administered orally on the indicated schedule. Therapy was initiated when tumor masses in the respective experiments reached 100−150 mg. Differences in treatment schedules indicate data from separate experiments. It is important to initiate therapy in different experiments at an equivalent tumor mass rather than fix the days of therapy. [b] Maximum therapy-induced weight loss. A net weight gain is indicated by a "+". [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 a fixed evaluation size of 750 mg. [e] Data from ref 15. [f] Maximum tolerated dose (≤LD_{10}). [g] HDT, highest dose tested.

The above results show that both *N*-methyl-substituted and *β*-substituted acrylamides retain good potency and ability for irreversible inhibition of EGFR and that the latter are suitable templates for the introduction of a wide range of solubility-enhancing groups.

In Vivo Studies. The *N*-methyl- and *β*-morpholinopropylbutenediamide analogues (**7** and **31**, respectively) were evaluated against A431 epidermoid, H125 non-small-cell lung, and MCF-7 estrogen-dependent breast xenografts in mice, and the results are given in Table 3. The *N*-methyl compound **7** was selected as it was the most active against the isolated EGFR enzyme, while **31** was selected for direct comparison with the clinical candidate **5** (same anilino ring substitution pattern). Both compounds proved ineffective against the A431 xenograft, while the clinical candidate **5** (CI-1033) was highly active. Neither **7** nor **31** showed any meaningful antitumor effects against the H125 or MCF-7 xenografts. Other than the weight loss noted in Table 3, there were no clinical signs of toxicity associated with the 200 mg/kg dosage level for either compound. The fact that 200 mg/kg doses were tolerated by the tumor-bearing animals may indicate that these compounds were not highly bioavailable when dosed as suspensions. The pyrido[3,4-*d*]pyrimidine **31** is clearly inferior[15,16] to the clinical evaluation compound **5**, a quinazoline which has the same anilino substitution pattern but bears the solubility-enhancing group separately off the 7-position, rather than off the end of the acrylamide. Since we have shown above and previously[14] that the chromophore has little effect, it appears that the positioning of the solubility-enhancing group is an important factor.

Conclusions

These results show that a range of Michael acceptors, apart from the unsubstituted acrylamide at the 6-position of 4-anilinoquinazolines and pyrido[3,4-*d*]pyrimidines, provide irreversible inhibitors of the EGFR enzyme. Of the non-acrylamide Michael acceptors studied, only the vinylsulfonamide provided comparably potent irreversible inhibitors, but these were less stable. Within the modified acrylamides, there was very limited bulk tolerance for substitution at the acrylamide nitrogen, with only the *N*-methyl analogue **7** retaining irreversible activity, and there was no tolerance at all for substitution at the acrylamide α-carbon (compounds **8/9**). In contrast, quite large electron-withdrawing groups (which increase acrylamide electrophilicity) were acceptable at the *β*-carbon. These amide-derived soluble analogues were potent irreversible inhibitors of isolated EGFR and effective inhibitors of both EGF-stimulated autophosphorylation of EGFR and heregulin-stimulated autophosphorylation of *erb*B2 in cellular assays, with activity profiles comparable to that of the clinical agent **5**. However, the best of these (**31**) was not nearly as active as **5** in vivo. Thus positioning the solubility-enhancing group off the *β*-carbon of the acrylamide may not be as useful as positioning it separately off the 7-position, since it may raise the general alkylating ability of the inhibitor.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or by the Analytical Department, Pfizer Global Research and Development, Ann Arbor Laboratories. Melting points were determined using an Electrothermal model 9200 or Gallenkamp digital melting point apparatus and are as read. NMR spectra were measured on Bruker AC-200 or AM-400 or Varian Unity 400-MHz spectrometers and referenced to Me_4Si. Mass spectra were recorded either on a Varian VG 7070 spectrometer at nominal 5000 resolution or on a Finnigan MAT 900Q spectrometer.

***N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-*N*-methylacrylamide (7): Example of Method of Scheme 1.** 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) (294 mg, 1.5 mmol) was added in one portion to a stirred solution of 4-(3-bromoanilino)-6-methylaminopyrido[3,4-*d*]pyrimidine[17] (**37**) (100 mg, 0.3 mmol), redistilled acrylic acid (75 μL, 1.05 mmol) and pyridine (0.3 mL) in THF/DMA (3:2, 1.8 mL) under N_2 at 0 °C. After 30 min the reaction was warmed to 25 °C, and after 3.75 h further acrylic acid (25 μL) was added. The mixture was stirred for an additional 3 h, then quenched with water. The precipitate was collected, air-dried, and triturated in hot CH_2Cl_2/EtOAc to give **7** (67 mg, 56%): mp 215−223 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.11 (s, 1 H, NH), 9.14 (s, 1 H), 8.80 (s, 1 H), 8.45 (s, 1 H), 8.22 (s, 1 H), 7.91 (br d, J = 7.7 Hz, 1 H), 7.43−7.36 (m, 2 H), 6.36−6.23 (m, 2 H), 5.66 (dd, J = 9.5, 3.0 Hz, 1 H), 3.44 (s, 3 H, CH_3); CIMS *m*/*z* (relative %) 383 (23), 384 (100), 385 (40), 386 (99), 387 (20). Anal. ($C_{17}H_{14}BrN_5O\cdot0.5H_2O$) C, N; H: found 3.5, calcd 4.1.

***N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-2-methylacrylamide (8): Example of Method of Scheme 1.** To a solution of 6-amino-4-(3-bromoanilino)pyrido[3,4-*d*]-pyrimidine[17] (**38**) (250 mg, 0.82 mmol), Et_3N (excess, 2.0 mL) and DMAP (catalytic) in THF (30 mL) under nitrogen was added methacryloyl chloride (88 μL, 0.90 mmol). The reaction was stirred at room temperature for 1.5 h over which time two further amounts (88 μL) of methacryloyl chloride were added. The reaction was then diluted with saturated $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and passed through a crude column of silica gel before preparative layer chromatography on silica gel eluting with EtOAc/CH_2Cl_2 (1:1), gave **8** (18 mg, 6%): mp (CH_2Cl_2/hexane) 177−178 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.61 (s, 1 H, NH), 10.29 (s, 1 H, NH), 9.06 (s, 1 H), 8.93 (s, 1 H), 8.67 (s, 1 H), 8.19 (t, J = 1.6 Hz, 1 H, H-2′), 7.91 (dt, J = 7.6, 1.6, 1.6 Hz, 1 H, H-6′), 7.38 (t, J = 7.9 Hz, 1 H, H-5′), 7.34 (dt, J = 8.1, 1.4, 1.4 Hz, 1 H, H-4′), 6.04 (s, 1 H, CH_2C(CH_3)CO), 5.64 (s, 1 H, CH_2C(CH_3)-CO), 2.03 (s, 1 H, CH_2C(CH_3)CO); HRMS (DEI) $C_{17}H_{14}{}^{81}BrN_5O$ requires 385.03613, found 385.03595.

***N*-[4-(3-Bromoanilino)quinazolin-6-yl]-2-methylacrylamide (9): Example of Method of Scheme 1.** To a stirred solution of 6-amino-4-(3-bromoanilino)quinazoline[18] (**39**) (0.50 g, 1.59 mmol) in THF (20 mL) under nitrogen were added Et_3N (excess, 1.0 mL), a catalytic amount of DMAP and methacryloyl chloride (171 μL, 1.75 mmol) dropwise. The reaction was stirred at room temperature for 1.5 h over which time two further amounts (50 μL) of methacryloyl chloride were added. Workup as above followed by chromatography on silica gel eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (5:45:50) and recrystallization from EtOAc gave **9** (195 mg, 32%): mp 244−245 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.15 (s, 1 H, NH), 9.90 (s, 1 H, NH), 8.80 (br s, 1 H, H-5), 8.60 (s, 1 H, H-2), 8.20 (br s, 1 H, H-2′), 7.97 (br d, J = 8.6 Hz, 1 H, H-7), 7.89 (br d, J = 7.7 Hz, 1 H, H-6′), 7.80 (d, J = 8.9 Hz, 1 H, H-8), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.30 (br d, J = 7.5 Hz, 1 H, H-4′), 5.94 (s, 1 H, CH_2C(CH_3)CO), 5.62 (s, 1 H, CH_2C(CH_3)-CO), 2.02 (s, 3 H, CH_2C(CH_3)CO). Anal. ($C_{18}H_{15}BrN_4O$) C, H, N.

(2*E*)-*N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-2-butenamide (10). EDCI·HCl (98 mg, 0.5 mmol) was added to a stirred solution of **38** (32 mg, 0.1 mmol) and *trans*-crotonic acid (35 mg, 0.4 mmol) in pyridine (0.4 mL) under N_2 at 0−5 °C. Cooling was removed and the mixture was stirred at 25 °C for 2 h, then diluted with water and the resulting suspension stirred for 15 min and filtered. The solid was dissolved in EtOAc, washed with 5% aqueous $NaHCO_3$, dried ($MgSO_4$) and filtered through a silica gel column. The filtrate was concentrated, and the resulting solid was triturated in hot EtOAc to give **10** (11 mg, 28%): mp >260 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.87 (s, 1 H, NH), 10.31 (s, 1 H, NH), 9·03 (s, 1 H), 9.00 (s, 1 H), 8.65 (s, 1 H), 8.17 (s, 1 H), 7.89 (d, J = 7.5 Hz, 1 H), 7.39−7.33 (m, 2 H), 6.99−6.90 (m, 1 H), 6.39 (dd, J = 15.4, 1.7 Hz, 1 H), 1.91 (dd, J = 7.0, 1.4 Hz, 3 H); MS (APCI) *m*/*z* (relative %) 381.8 (74), 382.8 (27), 383.8 (100), 384.8 (30), 385.9 (10). Anal. ($C_{17}H_{14}BrN_5O\cdot0.25H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Bromoanilino)-6-quinazolinyl]-2-butenamide (11). Excess *trans*-crotonyl chloride was added to a solution of **39** (316 mg, 1.0 mmol) in THF (6 mL) stirred under N_2 at 0 °C. After 2.5 h the resulting yellow solid was collected by filtration and sonicated with EtOAc to give **11** (216 mg, 52%): mp 279−281 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.55 (br s, 1 H, NH), 10.78 (s, 1 H, NH), 9.17 (d, J = 1.9 Hz, 1 H, H-5), 8.97 (s, 1 H, H-2), 8.12 (dd, J = 9.1, 2.0 Hz, 1 H, H-7), 8.05 (t, J = 1.9 Hz, 1 H, H-2′), 7.99 (d, J = 9.0 Hz, 1 H, H-8), 7.76 (dd, J = 8.1, 2.0 Hz, 1 H, H-6′), 7.58 (dd, J = 8.6, 1.7 Hz, 1 H, H-4′), 7.52 (t, J = 8.1 Hz, 1 H, H-5′) 7.03−6.94 (m, 1 H, (CO)CH=), 6.34 (dd, J = 15.1, 1.7 Hz, 1 H, CH=CHCH$_3$), 1.98 (dd, J = 6.8, 1.4 Hz, 3 H, CH_3); MS (CI) 385 (89, $^{81}BrMH^+$), 384 (51, $^{81}BrM^+$), 383 (100, $^{79}BrMH^+$), 382 (37, $^{79}BrM^+$). Anal. ($C_{18}H_{15}N_4$-BrO·HCl) C, H, N.

(2*Z*)-*N*-[4-(3-Bromoanilino)-6-pyrido[3,4-*d*]pyrimidin-6-yl]-3-chloro-2-propenamide (12). A stirred solution of **38** (128 mg, 0.4 mmol) and *cis*-3-chloroacrylic acid (172 mg, 1.6 mmol) in pyridine (2 mL) under N_2 was treated at −20 °C with EDCI·HCl (392 mg, 1.5 mmol). After 4.5 h at −20 °C, additional *cis*-3-chloroacrylic acid (57 mg) and EDCI·HCl (130 mg) were added, and the temperature was brought to −10 °C. After a total reaction time of 7 h, the viscous mixture was diluted with DMF and the resulting solution was poured into EtOAc/water (1:1). The aqueous phase was further extracted with EtOAc (2×), and the combined organic phases were washed with brine (2×), dried ($MgSO_4$) and filtered through a column of flash silica gel. The filtrate was concentrated to a

solid that was dissolved in warm EtOAc and chromatographed on flash silica gel, eluting with EtOAc. The appropriate fractions were pooled and evaporated. The product was triturated in EtOAc/*tert*-butyl methyl ether (1:1), dried at 0.1 mm/25 °C and recrystallized from EtOAc to give **12** (30 mg, 18%): mp 165−175 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.09 (s, 1 H, NH), 10.38 (s, 1 H, NH), 9·04 (s, 1 H), 9.00 (s, 1 H), 8.66 (s, 1 H), 8.16 (t, J = 1.9 Hz, 1 H), 7.88 (dt, J = 7.7, 1.7 Hz, 1 H), 7.40−7.33 (m, 2 H), 7.07 (d, J = 8.0 Hz, 1 H), 6.77 (d, J = 8.0 Hz, 1 H); MS (APCI) *m*/*z* (relative %) 365.8 (29), 366.8 (36), 367.8 (35), 368.8 (35), 401.8 (82), 402.8 (18), 403.8 (100), 404.8 (20), 405.8 (29). Anal. ($C_{16}H_{11}BrClN_5O·0.25C_4H_8O_2$) C, H, N.

(2*E*)-*N*-[4-(3-Bromoanilino)-6-quinazolinyl]-4,4,4-trifluoro-2-butenamide (13). A stirred solution of **39** (158 mg, 0.5 mmol) and 4,4,4-trifluorobut-2-enoic acid (153 mg, 1.1 mmol) in THF/DMF (4:1, 2.5 mL) was treated with EDCI·HCl (192 mg, 1.0 mmol) under N_2 at 0 °C. After 1 h the mixture was diluted with water (10 mL), and the resulting precipitate was collected, washed with water (2 × 5 mL) and ether (10 mL) and air-dried. The solid was suspended in EtOAc (10 mL), refluxed briefly, and sonicated for 10 min, then collected by filtration, washed with EtOAc (5 mL) and dried in a vacuum oven at 75 °C for 1.5 h to give **13** as the hydrochloride salt (76 mg, 33%): mp 273−278 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.09 (br s, 1 H, NH), 10.43 (s, 1 H, NH), 8.90 (s, 1 H, H-2), 8.70 (s, 1 H, H-5), 8.11 (s, 1 H, H-2′), 7.97 (dd, J = 2.5, 9.2 Hz, 1 H, H-7), 7.87 (d, J = 9.0 Hz, 1 H, H-8), 7.81 (d, J = 6.9 Hz, 1 H, H-6′), 7.41−7.33 (m, 2 H, H-5′,H-4′), 7,11 (d, J = 16.4 Hz, 1 H, CH═CHCF$_3$), 7.03 (dq, J_d = 16.4 Hz, J_q = 6.4 Hz, 1 H, CH═CHCF$_3$); MS (CI) 439 (78 ^{81}BrM$^+$), 437 (100 ^{79}BrM$^+$). Anal. ($C_{18}H_{13}BrF_3N_4O·0.5HCl$) C, H, N.

(2*E*)-*N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-2,4-pentadienamide (14). A stirred solution of **38** (160 mg, 0.5 mmol), 80% *trans*-2,4-pentadienoic acid (245 mg, 2 mmol), and pyridine (0.5 mL) in THF/DMA (2:1, 3 mL) under N_2 was cooled to 0−5 °C and treated with one portion of EDCI·HCl (490 mg, 2.5 mmol). Cooling was removed, and the viscous mixture was stirred at 25 °C for 23 h, then charged with additional *trans*-2,4-pentadienoic acid (125 mg), EDCI·HCl (240 mg) and THF/DMA (2:1, 2 mL). After a further 19 h the mixture was diluted with water and EtOAc. The biphasic mixture was warmed, then filtered through Celite, and the filter pad washed well with water and hot EtOAc. The filtrate was extracted with EtOAc (3×) and the combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated. The resulting solid was dissolved in hot EtOAc and chromatographed on silica gel, eluting with EtOAc. The product was triturated in warm EtOAc to give **14** (27 mg, 13%): mp 210−215 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.04 (s, 1 H, NH), 10.34 (s, 1 H, NH), 9.04 (s, 1 H), 9.02 (s, 1 H), 8.66 (s, 1 H), 8.17 (t, J = 1.9 Hz, 1 H), 7.89 (dt, J = 7.7, 1.7 Hz, 1 H), 7.40−7.27 (m, 3 H), 6.60 (dt, J = 16.9, 10.6 Hz, 1 H), 6.53 (d, J = 15.2 Hz, 1 H), 5.75 (d, J = 16.9 Hz, 1 H), 5.56 (d, J = 11.1 Hz, 1 H); MS (APCI) *m*/*z* (relative %) 395.9 (89), 396.9 (20), 397.9 (100), 398.9 (20). Anal. ($C_{18}H_{14}BrN_5O$) C, H.

***N*-[4-(3-Bromoanilino)-6-quinazolinyl]-2,3-butadienamide (15).** EDCI·HCl (384 mg, 2.0 mmol) was added to a stirred solution of **39** (316 mg, 1.0 mmol), and 2,3-butadienoic acid (173 mg, 2.06 mmol) in DMF (5 mL) stirred under N_2 at 0 °C. After 1.5 h the reaction was quenched with 0.1 M HCl (10 mL), and the resulting precipitate was collected and washed successively with water and Me_2CO, then dissolved into Me_2CO with the addition of Et_3N. The solution was filtered through a short column of silica gel in Me_2CO/CH_2Cl_2 (1:1) to give **15** (247 mg, 56%): mp 268−270 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.39 (s, 1 H, NH), 9.93 (s, 1 H, NH), 8.76 (d, J = 2.2 Hz, 1 H, H-5), 8.58 (s, 1 H, H-2), 8.18 (s, 1 H, H-2′), 7.87 (dt, J = 9.0, 1.9 Hz, 2 H, H-7,8), 7.79 (d, J = 8.8 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.29 (d, J = 8.3 Hz, 1 H, H-4′), 6.07 (t, J = 6.5 Hz, 1 H, C*H*═C═CH$_2$), 5.49 (d, J = 6.6 Hz, 2 H, ═C═CH$_2$); MS (APCI) 382.8 (88, ^{81}BrMH$^+$), 381.8 (19, ^{81}BrM$^+$), 380.7 (100, ^{79}BrMH$^+$). Anal. ($C_{18}H_{13}BrN_4O·0.8C_3H_6O·0.5H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-3-phenyl-2-propenamide (16). *trans*-Cinnamic acid (60 mg, 0.4 mmol) was added to a stirred solution of **38** (32 mg, 0.1 mmol) in pyridine (0.4 mL). EDCI·HCl (98 mg, 0.5 mmol) was added and the mixture was stirred under N_2 at 25 °C for 2 h. The mixture was diluted with water, and the solids were collected and dissolved in EtOAc. The solution was washed with 5% aqueous $NaHCO_3$, dried ($MgSO_4$) and filtered through silica gel. The residue from removal of solvent was triturated with hot EtOAc to give **16** (23 mg, 51%): mp 253−256 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.07 (s, 1 H, NH), 10.36 (s, 1 H, NH), 9.06 (s, 1 H), 9.02 (s, 1 H), 8.67 (s, 1 H), 8.19 (s, 1 H), 7.90 (d, J = 7.7 Hz, 1 H), 7.72−7.65 (m, 3 H), 7.51−7.34 (m, 5 H), 7.14 (d, J = 15.7 Hz, 1 H). Anal. ($C_{22}H_{16}N_5OBr·0.25H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Bromoanilino)-6-quinazolinyl]-4-oxo-2-pentenamide (17). *N*-Ethyldiisopropylamine (0.26 mL, 1.5 mmol) and **39** (0.23 g, 0.75 mmol) were added to a stirred solution of (*E*)-4-oxopent-2-enoic acid (171 mg, 1.5 mmol) and EDCI·HCl (288 mg, 1.5 mmol) in THF/DMF (3:1, 4 mL) under N_2 at 25 °C. The ice bath was then removed, and the reaction mixture was stirred at 25 °C for 4 h, when further *N*-ethyldiisopropylamine (0.13 mL, 0.75 mmol), (*E*)-4-oxopent-2-enoic acid (86 mg, 0.75 mmol) and EDCI·HC1 (144 mg, 0.75 mmol) were added. The reaction mixture was stirred for a further 14 h at 25 °C, then added dropwise to stirred cold water (100 mL). The solid was collected, dissolved in MeOH (50 mL) and evaporated onto silica gel (3 g). Flash chromatography on silica gel, eluting with 10% MeOH/CH_2Cl_2 (1:9), gave **17** (0.14 g, 45%): mp 230 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.91 (s, 1 H, NH), 9.99 (s, 1 H, NH), 8.87 (d, J = 1.9 Hz, 1 H, H-5), 8.60 (s, 1 H, H-2), 8.17 (t, J = 1.9 Hz, 1 H, H-2′), 7.85 (m, 3 H, H-7,8,6′), 7.37 (m, 2 H, H-5′,4′), 7.15 (d, J = 15.7 Hz, 1 H, pentenyl H-3), 6.99 (d, J = 15.7 Hz, 1 H, pentenyl H-2), 2.40 (s, 3 H, CH_3); MS (APCI) 412.7 (100, ^{81}BrMH$^+$), 410.8 (98, ^{79}BrMH$^+$). Anal. ($C_{19}H_{15}BrN_4O_2$) C, H, N.

(2*E*)-4-{[4-(3-Bromoanilino)-6-quinazolinyl]amino}-4-oxo-2-butenoic Acid (18). Maleic anhydride (0.266 g, 2.7 mmol) was added to a solution of **39** (0.78 g, 2.5 mmol) in DMF (8 mL), and the mixture was heated with stirring in a 70 °C oil bath for 2.5 h. The resulting suspension was cooled to room temperature and then diluted with water. The solid was collected, washed sequentially with a mixture of toluene/DMF (1:1), water, and IPA. The solid was dried under high vacuum at 60 °C for 16 h to give **18** (0.87 g, 86%): mp 224−225 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 13.00 (br s, 1 H, COOH), 10.85 (br s, 1 H, NH), 9.96 (br s, 1 H, NH), 8.73 (d, J = 1.8 Hz, 1 H, H-5), 8.54 (s, 1 H, H-2), 8.11 (br s, 1 H, $(CH_3)_2NCHO$), 7.91−7.75 (m, 4 H), 7.32−7.24 (m, 2 H), 6.46 (d, J = 12.0 Hz, 1 H, C*H*═CH), 6.35 (d, J = 12.0 Hz, 1 H, CH═C*H*), 2.84 (s, 3 H, $(CH_3)_2NCHO$), 2.68 (s, 3 H, $(CH_3)_2NCHO$); MS (APCI) 412.8 (100, ^{81}BrM$^+$), 410.8 (96, ^{79}BrM$^+$), 413.8 (26, ^{81}BrMH$^+$), 411.8 (24, ^{79}BrMH$^+$). Anal. ($C_{18}H_{13}BrN_4O_3·$DMF) C, H, N.

Ethyl (2*E*)-4-{[4-(3-Bromoanilino)-6-quinazolinyl]amino}-4-oxo-2-butenoate (19). *N*-Ethyldiisopropylamine (0.26 mL, 1.5 mmol) and **39** (0.23 g, 0.75 mmol) were added to a solution of (*E*)-4-ethoxy-4-oxobut-2-enoic acid (216 mg, 1.5 mmol) and EDCI·HC1 (288 mg, 1.5 mmol) in THF/DMF (3:1, 4 mL) stirred under N_2 at 25 °C. The ice bath was removed, and the reaction mixture was stirred at 25 °C for 4 h, when further *N*-ethyldiisopropylamine (0.13 mL, 0.75 mmol), (*E*)-4-ethoxy-4-oxobut-2-enoic acid (108 mg, 0.75 mmol), and EDCI·HC1 (144 mg, 0.75 mmol) were added. After stirring a further 14 h at 25 °C, the reaction mixture was added dropwise to stirred cold water (100 mL). The solid was collected, dissolved in MeOH (50 mL), and dried onto silica gel (3 g) and flash chromatographed on silica gel, eluting with MeOH/CH_2C1_2 (1:9). Concentration of pure fractions under reduced pressure gave **19** (0.19 g, 58%): mp >255 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.93 (s, 1 H, NH), 9.99 (s, 1 H, NH), 8.89 (d, J = 1.9 Hz, 1 H, H-5), 8.60 (s, 1 H, H-2), 8.16 (t, J = 1.9 Hz, 1 H, H-2′), 7.85 (m, 3 H, H-7,8,6′), 7.33 (m, 3 H, H-5′,4′, pentenyl H-3), 6.79 (d, J = 15.4 Hz, 1H, pentenyl H-2), 4.24 (q, J = 7.1 Hz, 2 H, CH_2), 1.29 (t, J = 7.1 Hz, 3 H, CH_3); MS (APCI) 442.8 (99, ^{81}BrMH$^+$), 440.8 (100, ^{79}BrMH$^+$). Anal. ($C_{20}H_{17}BrN_4O_3$) C, H, N.

Ethyl (2*E*)-4-{[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-4-oxo-2-butenoate (20). Reaction of **38** (32 mg, 0.1 mmol) with fumaric acid monoethyl ester (58 mg, 0.4 mmol) and EDCI·HCl (98 mg, 0.5 mmol) in pyridine (0.5 mL) was carried out as described above. After 5 h, the solution was poured into water, which formed a precipitate. The suspension was sonicated, then the solid was collected, washed well with water, and dried to give **20** (90 mg, 89%): mp >230 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.44 (s, 1 H, NH), 10.37 (s, 1 H, NH), 9.07 (s, 1 H, H-2), 9.05 (s, 1 H, H-8), 8.68 (s, 1 H, H-5), 8.17 (d, J = 2.0 Hz, 1 H, H-2′), 7.89 (dt, J = 7.5, 1.9, 1.7 Hz, 1 H, H-4′), 7.48 (d, J = 15.4 Hz, 1 H, fumarate H), 7.40−7.34 (m, 2 H, H-5′,6′), 6.83 (d, J = 15.4 Hz, 1 H, fumarate H), 4.24 (q, J = 7.0 Hz, 2 H, CH_2), 1.28 (t, J = 7.0 Hz, 3 H, CH_3). Anal. ($C_{19}H_{16}BrN_5O_3$·0.25H_2O) C, H, N.

***N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-*N*-[2-(dimethylamino)ethyl]acrylamide (21): Example of Method of Scheme 2.** EDCI·HCl (980 mg, 5 mmol) was added to a stirred solution of N^4-(3-bromophenyl)-N^6-[2-(dimethylamino)ethyl]pyrido[3,4-*d*]pyrimidine-4,6-diamine[19] (**40**) (387 mg, 1 mmol) and redistilled acrylic acid (0.25 mL, 3.6 mmol) in pyridine (5 mL) under N_2 cooled to 0−5 °C. After 30 min cooling was removed, and the solution was stirred for an additional 45 min, then diluted with 1% aqueous $NaHCO_3$. The mixture was extracted with EtOAc (4×), and the combined extracts were washed with brine, dried ($MgSO_4$), and concentrated to give **21** (122 mg, 28%): mp (EtOAc, 5 °C) >160 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.16 (s, 1 H, NH), 9.15 (s, 1 H), 8.80 (s, 1 H), 8.43 (s, 1 H), 8.22 (s, 1 H), 7.93 (d, J = 7.7 Hz, 1 H), 7.42−7.35 (m, 2 H), 6.29−6.22 (m, 2 H), 5.66 (dd, J = 9.0, 3.5 Hz, 1 H), 4.05 (t, J = 7.1 Hz, 2 H) 2.42 (t, J = 7.1 Hz, 2 H), 2.11 (s, 6 H); MS (APCI) *m*/*z* (relative %) 440.9 (99), 441.8 (23), 442.8 (100), 443.9 (24). Anal. ($C_{20}H_{21}BrN_6O$) C, H, N.

***N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-*N*-[3-(4-morpholinyl)propyl]acrylamide (22): Example of Method of Scheme 2.** To a stirred solution of N^4-(3-bromophenyl)-N^6-[3-(4-morpholinyl)propyl]pyrido[3,4-*d*]pyrimidine-4,6-diamine[19] (**41**) (400 mg, 0.90 mmol), DMAP (40 mg) and Et_3N (excess, 2.0 mL) at 0 °C under N_2 was added acryloyl chloride (89 μL, 1.08 mmol). After 1 h stirring a further two portions of acid chloride (89 μL each) were added over the next 2 h and the procedure and workup above were followed to give, after column chromatography on silica gel eluting with MeOH/EtOAc (1:9) to MeOH/EtOAc (1:5), **22** (142 mg, 32%): mp (CH_2Cl_2/hexane) 178−180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.15 (s, 1 H, NH), 9.15 (s, 1 H), 8.80 (s, 1 H), 8.47 (s, 1 H), 8.21 (br s, 1 H, H-2′), 7.92 (br d, J = 7.6 Hz, 1 H, H-6′), 7.41 (t, J = 8.0 Hz, 1 H, H-5′), 7.37 (dt, J = 8.1, 1.6, 1.6 Hz, 1 H, H-4′), 6.25 (m, 2 H, CH_2CHCO, CH_2CHCO), 5.66 (m, 1 H, CH_2CHCO), 3.98 (t, J = 7.5 Hz, 2 H, CH_2NRCO), 3.46 (t, J = 4.5 Hz, 4 H, morph. CH_2), 2.29 (t, J = 7.1 Hz, 2 H, $CH_2CH_2CH_2NRCO$), 2.24 (br s, 4 H, morph. CH_2), 1.73 (quintet, J = 7.2 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{23}H_{25}BrN_6O_2$·H_2O) C, H, N.

***N*-[4-(3-Bromoanilino)-6-quinazolinyl]-*N*-[3-(4-morpholinyl)propyl]acrylamide (23): Example of Method of Scheme 3.** A stirred solution of **4**[14] (1.78 g, 4.82 mmol), morpholine (excess, 4.0 mL) and *p*-toluenesulfonic acid (catalytic) in THF (50 mL) was heated at 50 °C for 4 h before being concentrated under reduced pressure, diluted with water and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and chromatographed on silica gel eluting with MeOH/CH_2Cl_2/EtOAc (15:40:45) to give *N*-[4-(3-bromoanilino)-6-quinazolinyl]-3-(4-morpholinyl)propanamide (**42**) (1.86 g, 78%): mp (EtOAc) 184−186 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.37 (s, 1 H, NH), 9.91 (s, 1 H, NH), 8.72 (d, J = 1.9 Hz, 1 H, H-5), 8.58 (s, 1 H, H-2), 8.17 (t, J = 2.1 Hz, 1 H, H-2′), 7.86 (m, 2 H, H-7, 6′), 7.78 (d, J = 8.9 Hz, 1 H, H-8), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 1.2, 1.2, 8.0 Hz, 1 H, H-4′), 3.40 (t, J = 4.6 Hz, 4 H, morph. CH_2), 2.69 (t, J = 6.6 Hz, 2 H, NCH_2CH_2CONH), 2.58 (t, J = 6.6 Hz, 2 H, NCH_2CH_2CONH), 2.44 (br s, 4 H, morph. CH_2). Anal. ($C_{21}H_{22}BrN_5O_2$) C, H, N.

To a stirred solution of **42** (0.85 g, 1.86 mmol) in THF (30 mL) under N_2 at 0 °C was added BH_3·DMS (372 μL of a 10M solution, 2 mol equiv) dropwise. The resulting solution was allowed to warm to room temperature and then stirred for 2 h, before being quenched by the cautious addition of 1 N HCl (40 mL). The reaction mixture was then stirred at 50 °C for 2 h, basified by the addition of saturated Na_2CO_3 and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and chromatographed on silica gel eluting with MeOH/CH_2Cl_2/EtOAc (3:8:8) to give N^4-(3-bromophenyl)-N^6-[3-(4-morpholinyl)propyl]-4,6-quinazolinediamine (**43**) (130 mg, 16%) as a yellow glass (ca. 90% pure by NMR). This was used without further purification: ^{1}H NMR [$(CD_3)_2SO$] δ 9.40 (s, 1 H, NH), 8.37 (s, 1 H, H-2), 8.17 (t, J = 1.9 Hz, 1 H, H-2′), 7.91 (br d, J = 8.2 Hz, 1 H, H-6′), 7.54 (d, J = 9.0 Hz, 1 H, H-8), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.27 (m, 2H, H-4′, 7), 7.16 (d, J = 2.2 Hz, 1 H, H-5), 6.25 (t, J = 5.1 Hz, 1 H, CH_2NH), 3.59 (t, J = 4.5 Hz, 4 H, morph. CH_2), 3.22 (q, J = 6.0 Hz, 1 H, CH_2NH), 2.45 (t, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2NH$), 2.39 (br s, 4 H, morph. CH_2), 1.82 (quintet, J = 7.0 Hz, 2 H, $CH_2CH_2CH_2$).

A stirred solution of **43** (133 mg, 0.30 mmol) in DMF (5.0 mL) under N_2 was treated sequentially with acrylic acid (83 μL, 1.20 mmol), Et_3N (excess, 0.5 mL), and EDCI·HCl (115 mg, 0.6 mmol). Standard workup as above, followed by chromatography on silica gel, eluting with EtOAc:CH_2Cl_2 (1:1) to MeOH/CH_2Cl_2/EtOAc (3:7:10), gave **23** (39 mg, 26%): mp (CH_2Cl_2/hexane) 171−175 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.86 (s, 1 H, NH), 8.70 (s, 1 H, H-2), 8.52 (d, J = 2.0 Hz, 1 H, H-5), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.91 (partially obscured br d, J = 8.6 Hz, 1 H, H-6′), 7.89 (d, J = 8.9 Hz, 1 H, H-8), 7.79 (dd, J = 8.8, 2.1 Hz, 1 H, H-7), 7.38 (t, J = 7.9 Hz, 1 H, H-5′), 7.33 (dt, J = 8.4, 1.7, 1.7 Hz, 1 H, H-4′), 6.22 (dd, J = 16.7, 2.3 Hz, 1 H, CH_2CHCO), 6.05 (br s, 1 H, CH_2CHCO), 5.61 (br d, J = 8.8 Hz, 1 H, CH_2CHCO), 3.87 (t, J = 7.4 Hz, 2 H, CH_2NRCO), 3.49 (t, J = 4.5 Hz, 4 H, morph. CH_2), 2.28 (partially obscured t, J = 7.1 Hz, 2 H, $CH_2CH_2CH_2NRCO$), 2.27 (br s, 4 H, morph. CH_2), 1.69 (quintet, J = 7.3 Hz, 2 H, $CH_2CH_2CH_2$); HRMS (DEI) (M^+) calcd for $C_{24}H_{26}Br^{81}N_5O_2$ 497.1249, found 497.1250.

3-(Dimethylamino)propyl (2*E*)-4-{[4-(3-Bromoanilino)-6-quinazolinyl]amino}-4-oxo-2-butenoate (24): Example of Method of Scheme 4. A solution of **39** (158 mg, 0.5 mmol) in THF (10 mL) was added dropwise over 15 min to a solution of fumaryl chloride (382 mg, 2.5 mmol) in THF (10 mL) stirred under N_2 at 0 °C. After 1 h at 0 °C the suspension was allowed to settle, and the supernatant of crude acid chloride **44** was decanted. Fresh THF (5 mL) was added, and the suspension was stirred at 0 °C while a solution of 3-(*N*,*N*-dimethylamino)-propan-l-ol (1.18 mL, 10 mmol) in THF (5 mL) was added dropwise. The suspension was stirred at 25 °C for 1 h, the solvent was evaporated under reduced pressure, and the residue was triturated with cold water. The solid was collected, dissolved in a minimum volume of DMF, and absorbed onto silica gel (2 g) and dried. Flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (2:1) gave a product that was dissolved in AcOH/water (3:2, 2.5 mL), passed through a 0.45-μm filter, and purified by HPLC on a Vidac C_{18} 218TP1022 reverse-phase HPLC column. Elution with 10% to 50% gradient of 0.1% TFA in water/0.l % TFA in CH_3CN over 60 min gave **24** as the tris trifluoroacetate salt (51 mg, 12%): mp 60 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.14 (s, 1 H, NH), 10.85 (br s, 1 H, NH), 9.57 (br s, 1 H, NH), 9.01 (d, J = 1.7 Hz, 1 H, H-5), 8.79 (s, 1 H, H-2), 8.07 (s, 1H, H-2′), 8.02 (dd, J = 2.1, 9.0 Hz, 1H, H-7), 7.89 (d, J = 8.9 Hz, 1H, H-8), 7.78 (d, J = 6.5 Hz, 1 H, H-6′), 7.43 (m, 2 H, H-4′,5′), 7.34 (d, J = 15.4 Hz, 1 H, butenyl H-3), 6.84 (d, J = 15.4 Hz, 1 H, butenyl H-2), 4.26 (t, J = 6.2 Hz, 2 H, OCH_2), 3.19 (m, 2 H, CH_2N), 2.81 (d, J = 4.6 Hz, 6 H, CH_3), 2.05 (m, 2 H, CH_2); MS (APCI) 499.8 (100, $^{81}BrMH^+$), 497.9 (97, $^{79}BrMH^+$). Anal. ($C_{23}H_{24}BrN_5O_3$·$3CF_3CO_2H$) C, H, N.

(2*E*)-N^1-[4-(3-Bromoanilino)-6-quinazolinyl]-N^4-[3-(dimethylamino)propyl]-2-butenediamide (25). A solution of **39** (158 mg, 0.5 mmol) in THF (10 mL) was added dropwise

over 15 min to a solution of fumaryl chloride (382 mg, 2.5 mmol) in THF (10 mL) stirred under N_2 at 0 °C. After 1 h at 0 °C, the suspension was allowed to settle, and the supernatant was decanted. Fresh THF (5 mL) was added and the suspension was stirred at 0 °C while a solution of N^1,N^1-dimethyl-1,3-propanediamine (1.26 mL, 10 mmol) in THF (5 mL) was added dropwise. The suspension was stirred at 25 °C for 1 h, the solvent was stripped under reduced pressure, and the residue was triturated with cold water. The solid was collected, dissolved in boiling MeOH (25 mL), filtered, and evaporated under reduced pressure. The residue was dissolved in AcOH/water (3:2, 2.5 mL) and purified by HPLC on a Vidac C_{18} 218TP1022 reverse-phase HPLC column. Elution with a 10% to 50% gradient of 0.1% TFA in water/0.l% TFA in CH_3CN over 60 min gave **25** as the tris trifluoroacetate salt (154 mg, 37%): mp 40 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.02 (s, 1 H, NH), 9.50 (br s, 1 H, NH) 9.02 (d, J = 1.7 Hz, 1 H, H-5), 8.82 (s, 1 H, H-2), 8.74 (t, J = 5.7 Hz, 1 H, NH), 8.05 (s, 1 H, H-2′), 8.02 (dd, J = 2.1, 9.0 Hz, 1 H, H-7), 7.89 (d, J = 8.9 Hz, 1 H, H-8), 7.76 (d, J = 7.2 Hz, H-6′), 7.45 (m, 2 H, H-4′,5′), 7.17 (d, J = 14.9 Hz, 1 H, butenyl H-3), 7.05 (d, J = 15.2 Hz, 1 H, butenyl H-2), 3.03 (m, 2 H, NCH_2), 3.08 (m, 2 H, CH_2N), 2.79 (d, J = 4.8 Hz, 6 H, CH_3), 1.83 (m, 2 H, CH_2); MS (APCI) 498.8 (100, $^{81}BrMH^+$), 496.9 (97, $^{79}BrMH^+$). Anal. ($C_{23}H_{25}BrN_6O_2 \cdot 3CF_3CO_2H$) C, H, N.

(2*E*)-N^1-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-N^4-[3-(4-morpholinyl)propyl]-2-butenediamide (28): Example of Method of Scheme 5.[20] A solution of (*E*)-but-2-enedioic acid monoethyl ester (**45**) (5.77 g 40.0 mmol) in THF (40 mL) was treated with oxalyl chloride (7.0 mL, 80.0 mmol), followed by two drops of DMF. The solution was stirred at room temperature for 1 h, then concentrated. The resulting residue was dissolved in CH_2Cl_2 (50 mL) and added dropwise during 20 min to a solution of 3-morpholin-4-ylpropylamine (6.43 mL, 44.0 mmol) in CH_2Cl_2 (200 mL) in a dry ice/acetone bath. At the end of the addition the reaction was allowed to come to room temperature, then poured into of 5% aqueous $NaHCO_3$ (500 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried ($MgSO_4$) and concentrated. Flash chromatography over silica gel, eluting with MeOH/CH_2Cl_2 (1:5) gave ethyl (2*E*)-4-{[3-(4-morpholinyl)propyl]amino}-4-oxo-2-butenoate (**46a**) (8.44 g, 78%) as an oil: ^{1}H NMR [$(CD_3)_2SO$] δ 8.53 (t, J = 5.5 Hz, 1 H, NH), 7.76 (d, J = 15.4 Hz, 1 H, olefinic), 6.55 (d, J = 15.4 Hz, 1 H, olefinic), 4.20 (q, 2 H, J = 7.0 Hz, C*H*$_2$CH$_3$), 3.56 (t, J = 4.7 Hz, 4 H, morph. CH_2), 3.18 (q, 2 H, C*H*$_2$NH, coalesces to t on D_2O wash), 2.32 (br s, 4 H, morph. CH_2), 2.28 (t, J = 7.2 Hz, 2 H, morpholino-C*H*$_2$CH$_2$CH$_2$), 1.59 (quintet, J = 7.0 Hz, 2 H, CH$_2$C*H*$_2$CH$_2$), 1.24 (t, J = 7.1 Hz, 3 H, CH_3); MS (APCI) M + 1 calcd 271.2, found 271.1.

A solution of **46a** (0.50 g, 1.8 mmol) and Et_3N (0.50 mL, 3.6 mmol) in water (10 mL) was stirred at room temperature overnight, concentrated, and coevaporated with EtOH to give crude (2*E*)-4-{[3-(4-morpholinyl)propyl]amino}-4-oxo-2-butenoic acid (**47a**) as a gum (containing Et_3N): ^{1}H NMR [$(CD_3)_2SO$] δ 8.41 (t, J = 5.3 Hz, 1 H, NH), 6.81 (d, J = 15.7 Hz, 1 H, olefinic), 6.49 (d, J = 15.4 Hz, 1 H, olefinic), 3.56 (t, J = 4.6 Hz, 4 H, morph. CH_2), 3.16 (dd, J = 12.8, 7.0 Hz, 2 H, C*H*$_2$-NH, coalesces to t on D_2O wash), 2.32 (br s, 4 H, morph. CH_2), 2.27 (t, J = 7.1 Hz, 2 H, morpholino-C*H*$_2$CH$_2$CH$_2$), 1.59 (quintet, J = 7.0 Hz, 2 H, CH$_2$C*H*$_2$CH$_2$); MS (APCI) M + 1 calcd 243.1, found 243.2.

A mixture of **47a** (assumed 1.8 mmol) and amine **38** (0.10 g, 0.32 mmol) in pyridine (2 mL) was treated with EDCI·HCl (0.34 g, 1.8 mmol), and the solution was stirred at room temperature overnight. The reaction was then poured into water, and the resulting solid was purified by flash chromatography over silica gel, eluting with MeOH/CH_2Cl_2 (1:4) to give **28** (54 mg, 30.0%): mp 237−240 °C dec; ^{1}H NMR [$(CD_3)_2$-SO] δ 11.3 (s, 1 H, NH), 10.4 (s, 1 H, NH), 9.05 (s, 1 H), 9.02 (s, 1 H), 8.67 (s, 1 H), 8.58 (t, J = 5.5 Hz, 1 H, CH$_2$N*H*), 8.17 (d, J = 1.7 Hz, 1 H), 7.89 (d, J = 6.5 Hz, 1 H), 7.36 (m, 2 H), 7.27 (d, J = 15.2 Hz, 1 H, olefinic), 7.08 (d, J = 15.2 Hz, 1 H, olefinic), 3.57 (t, J = 4.6 Hz, 4 H, morph. CH_2), 3.21 (dd, J = 12.7, 6.6 Hz, 2 H, C*H*$_2$NH, coalesces to t with D_2O), 2.34 (br s, 4 H, morph. CH_2), 2.31 (t, J = 7.2 Hz, 2 H, morpholino-C*H*$_2$-CH$_2$CH$_2$), 1.63 (quintet, J = 7 Hz, 2 H, CH$_2$C*H*$_2$CH$_2$); MS (APCI) M + 1 calcd 540.1, found 540.2. Anal. ($C_{24}H_{26}BrN_7O_3 \cdot 1.5H_2O$) C, H, N.

(2*E*)-N^1-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-N^4-[3-(dimethylamino)propyl]-2-butenediamide (26). Similar reaction of **45** (5.75 g, 40 mmol), oxalyl chloride (7 mL) and DMF (3 drops) gave the crude acid chloride. This was diluted with anhydrous Et_2O (200 mL) and the solution was cooled to −78 °C with mechanical stirring. The rapidly stirring solution was then treated dropwise with a solution of 3-dimethylaminopropylamine (5.54 mL, 44 mmol) in Et_2O (60 mL). After addition was completed, the bath was removed and the thick suspension was allowed to slowly warm to room temperature over 1−2 h. The solid was collected and washed well with ether. It was then dissolved in water (orange solution), and the pH was adjusted to ca. 10.5 with sodium carbonate. The aqueous phase was extracted with EtOAc (4×), then the combined extracts were washed with brine, dried, and filtered through a pad of flash silica gel. The filtrate was concentrated to leave crude ethyl (2*E*)-4-{[3-(dimethylamino)propyl]amino}-4-oxo-2-butenoate (**46b**) (2.75 g, 30%; low yield was likely due to incomplete extraction due to water solubility), sufficiently pure by NMR to use in the next reaction: ^{1}H NMR ($CDCl_3$) δ 7.94 (br s, 1 H, NH), 6.80 (d, J = 15.6 Hz, 1 H, olefinic), 6.73 (d, J = 15.6 Hz, 1 H, olefinic), 4.20 (q, J = 7.1 Hz, 2 H, C*H*$_2$-CH$_3$), 3.42 (q, J = 5.8 Hz, 2 H, C*H*$_2$NH), 2.43 (t, J = 5.8 Hz, 2 H, Me$_2$NC*H*$_2$CH$_2$CH$_2$), 2.24 (s, 6 H, N(CH$_3$)$_2$), 1.68 (quintet, J = 5.8 Hz, 2 H, CH$_2$C*H*$_2$CH$_2$), 1.27 (t, J = 7.1 Hz, 3 H, CH_3).

A solution of **46b** (2.43 g, 10.6 mmol) in deionized water (40 mL) was heated at reflux for 4 h. The water was stripped off and the residue was coevaporated with MeOH. The oil was dissolved in ca. 20 mL of MeOH, then treated with excess 2-propanolic HCl. The resultant solution was concentrated, the resulting solid was triturated in EtOH, washed with EtOH and dried to give (2*E*)-4-{[3-(dimethylamino)propyl]amino}-4-oxo-2-butenoic acid (**47b**) as the HCl salt (1.13 g): mp 148−152 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.77 (t, J = 5.8 Hz, 1 H, NH), 6.92 (d, J = 15.7 Hz, 1 H, olefinic), 6.53 (d, J = 15.7 Hz, 1 H, olefinic), 3.23 (q, J = 6.5 Hz, 2 H, C*H*$_2$NH, coalesces to t on D_2O wash), 3.03 (m, 2 H, Me$_2$NC*H*$_2$CH$_2$CH$_2$), 2.72 (s, 6 H, N(CH$_3$)$_2$), 1.84 (quintet, J = 7.0 Hz, 2 H, CH$_2$C*H*$_2$CH$_2$). Anal. ($C_9H_{16}N_2O_3 \cdot HCl$) C, H, N. Concentration of the filtrate gave a second crop (573 mg; total yield 1.7 g (68%)).

A suspension of **38** (64 mg, 0.2 mmol) and **47b** HCl salt (190 mg, 0.8 mmol) in pyridine (1 mL) was mixed by warming, then cooled in an ice bath and treated with EDCI·HCl (196 mg). The resulting suspension was stirred under nitrogen at 0−5 °C for 2.5 h, then diluted with DMF (2 mL). The solution was added to a 1:1 mixture of water/EtOAc, and shaken until phase separation. The aqueous phase was further extracted with EtOAc (3×) and the combined extracts were discarded. The aqueous layer was made basic with 5% aqueous $NaHCO_3$, and then extracted with EtOAc (5×) using small amounts of MeOH each time if necessary to bring about phase separation. The EtOAc extracts were combined and washed with brine, dried ($MgSO_4$), and concentrated to leave a residue. This was sonicated in 2-propanol to give **26** (67 mg, 66%): mp 235−38 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.3 (s, 1 H, NH), 10.4 (s, 1 H, NH), 9.05 (s, 1 H), 9.02 (s, 1 H), 8.67 (s, 1 H), 8.57 (t, J = 5.5 Hz, 1 H, CH$_2$N*H*), 8.17 (br s, 1 H), 7.88 (d, J = 7.5 Hz, 1 H), 7.36 (m, 2 H), 7.27 (d, J = 15.2 Hz, 1 H, olefinic), 7.07 (d, J = 15.2 Hz, 1 H, olefinic), 3.20 (q, J = 7.0 Hz, 2 H, C*H*$_2$NH, coalesces to t with D_2O), 2.23 (t, J = 7.0 Hz, 2 H, Me$_2$NC*H*$_2$CH$_2$CH$_2$), 2.13 (s, 6 H, N(CH$_3$)$_2$), 1.59 (quintet, J = 7.0 Hz, 2 H, CH$_2$C*H*$_2$-CH$_2$). Anal. ($C_{22}H_{24}BrN_7O_2 \cdot 0.5H_2O$) C, H, N.

(2*E*)-N^1-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-N^4-[3-(diethylamino)propyl]-2-butenediamide (27). Similar reaction of the acid chloride of **45** (from 5.75 g of **45**) with 3-diethylaminopropylamine (7.0 mL, 44.4 mmol) in CH_2Cl_2 (200 mL) gave ethyl (2*E*)-4-{[3-(diethylamino)propyl]amino}-4-oxo-2-butenoate (**46c**) (4.07 g, 37%) as an oil: ^{1}H NMR ($CDCl_3$) δ 8.47 (br s, 1 H, NH), 6.84 (d, J = 15.4 Hz, 1 H,

olefinic), 6.77 (d, J = 15.4 Hz, 1 H, olefinic), 4.24 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 3.48 (q, J = 5.5 Hz, 2 H, CH_2NH), 2.63 (m, 6 H, $Et_2NCH_2CH_2CH_2$, $N(CH_2CH_3)_2$), 1.75 (quintet, J = 5.5 Hz, 2 H, $CH_2CH_2CH_2$), 1.31 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.10 (t, J = 7.1 Hz, 6 H, $N(CH_2CH_3)_2$). Anal. ($C_{13}H_{24}N_2O_3 \cdot H_2O$) C, H, N.

A solution of **46c** (4.05 g, 14.86 mmol) and LiOH monohydrate (1.27 g, 30.1 mmol) in MeOH:H_2O (100 mL) was stirred at room temperature for 2 h. The mixture was evaporated to a semisolid, then diluted with warm MeOH and filtered through a pad of flash silica gel, washing well with MeOH. The eluate was concentrated to a solid that was triturated in Et_2O to give (2*E*)-4-{[3-(dimethylamino)propyl]amino}-4-oxo-2-butenoic acid (**47c**) as the lithium salt (3 g). This salt (2.55 g, 10 mmol) was dissolved in water and the pH was adjusted to 4 with dilute aqueous HCl. This solution was loaded onto a column packed with 10 g dry weight of Dowex 50W x 8 resin (H^+ form; 200−400 mesh; 5.2 mol equiv/g), and the column was eluted successively with 50 mL each of water, 0.1 M aqueous NH_3, 0.2 M aqueous NH_3, and 100 mL 0.3 M aqueous NH_3. Product fractions were pooled and concentrated to ca. 100 mL of solution that was then lyophilized and further dried at 5 mm/60 °C/5 h to provide the free acid (1.82 g) as a glassy solid that was used directly in the next reaction: ^{1}H NMR [$(CD_3)_2SO$] δ 8.46 (t, J = 5.3 Hz, 1 H, NH), 6.74 (d, J = 15.4 Hz, 1 H, olefinic), 6.48 (d, J = 15.4 Hz, 1 H, olefinic), 3.16 (q, J = 5.8 Hz, 2 H, CH_2NH, coalesces to t on D_2O wash), 2.69 (m, 6 H, $Et_2NCH_2CH_2CH_2$, $N(CH_2CH_3)_2$), 1.66 (quintet, J = 7.0 Hz, 2 H, $CH_2CH_2CH_2$), 1.04 (t, J = 7.2 Hz, 6 H, $N(CH_2CH_3)_2$).

Reaction of **38** (32 mg, 1 mmol), **47c** (97 mg, 0.4 mmol), EDCI·HCl (98 mg) and pyridine (0.5 mL) was carried out as described above. After 1.25 h further EDCI·HCl (45 mg) was added, and the mixture was stirred at room temperature for an additional 17 h. Workup as above, followed by trituration in 2-propanol, gave **27** (10.5 mg, 20%): mp 227−232 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.3 (s, 1 H, NH), 10.4 (s, 1 H, NH), 9.05 (s, 1 H), 9.02 (s, 1 H), 8.67 (s, 1 H), 8.56 (t, J = 5.5 Hz, 1 H, CH_2NH), 8.17 (br s, 1 H), 7.88 (d, J = 7.5 Hz, 1 H), 7.37 (m, 2 H), 7.27 (d, J = 15.2 Hz, 1 H, olefinic), 7.08 (d, J = 15.2 Hz, 1 H, olefinic), 3.20 (q, J = 6.0 Hz, 2 H, CH_2NH, coalesces to t with D_2O), 2.44 (m, 6 H, $Et_2NCH_2CH_2CH_2$, $N(CH_2CH_3)_2$), 1.57 (quintet, J = 7.0 Hz, 2 H, $CH_2CH_2CH_2$), 0.94 (t, J = 7.2 Hz, 6 H, $N(CH_2CH_3)_2$). Anal. ($C_{24}H_{28}BrN_7O_2$) C, H.

(2*E*)-*N*^{1}-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-*N*^{4}-[3-(1*H*-imidazol-1-yl)propyl]-2-butenediamide (29). Similar reaction of the acid chloride of **45** (from 5.77 g of **45**) with a solution of 3-imidazol-1-ylpropylamine (5.25 mL, 44.0 mmol) in CH_2Cl_2 (200 mL) in a dry ice/acetone bath, followed by flash chromatography of the residue on silica gel, eluting with MeOH/CH_2Cl_2 (1:2), gave ethyl (2*E*)-4-{[3-(imidazol-1-yl)-propyl]amino}-4-oxo-2-butenoate (**46d**) (5.21 g, 52%), which solidified on standing: ^{1}H NMR [$(CD_3)_2SO$] δ 8.60 (t, J = 5.4 Hz, 1 H, NH), 7.62 (s, 1 H, imidazole H), 7.18 (t, J = 1.2 Hz, 1 H, imidazole H), 6.99 (d, J = 15.7 Hz, 1 H, olefinic), 6.89 (s, 1 H, imidazole H), 6.57 (d, J = 15.7 Hz, 1 H, olefinic), 4.19 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 3.98 (t, J = 7.0 Hz, 2 H, CH_2N), 3.11 (q, J = 6.8 Hz, 2 H, CH_2NH, coalesces to t with D_2O), 1.88 (quintet, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2$), 1.24 (t, J = 7.0 Hz, 3 H, CH_3); MS (APCI) M + 1 calcd 252.1, found 252.1. Anal. ($C_{12}H_{17}N_3O_3$) C, H, N.

A solution of **46d** (1.27 g, 5.05 mmol) and Et_3N (1.42 mL, 10.2 mmol) in deionized water (25.5 mL) was heated at 60 °C for 30 min. The water was evaporated and residue was coevaporated with MeOH. The residue was crystallized from MeOH/2-propanol (1:1) to give (2*E*)-4-{[3-(imidazol-1-yl)propyl]-amino}-4-oxo-2-butenoic acid (**47d**) (190 mg, 17%): mp 170−174 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.55 (t, J = 5.5 Hz, 1 H, NH), 7.64 (s, 1 H, imidazole H), 7.19 (t, J = 1.2 Hz, 1 H, imidazole H), 6.90 (d, J = 15.4 Hz, 1 H, olefinic), 6.89 (s, 1 H, imidazole H), 6.52 (d, J = 15.4 Hz, 1 H, olefinic), 3.98 (t, J = 7.0 Hz, 2 H, CH_2N), 3.11 (q, J = 6.8 Hz, 2 H, CH_2NH, coalesces to t with D_2O), 1.88 (quintet, J = 6.8 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{10}H_{13}N_3O_3$) C, H, N. Further processing of the filtrate afforded 420 mg (37%) of additional product (total yield 610 mg (54%)).

Reaction of **47d** (156 mg, 0.7 mmol) and **38** (64 mg, 0.2 mmol) with EDCI·HCL (196 mg) in pyridine (1 mL) was carried out as described above. After 2 h the dark orange solution was diluted with DMF (2 mL) and poured into 2.5% aqueous $NaHCO_3$. The mixture was extracted with EtOAc (5×), and the combined EtOAc extracts were washed with brine, dried ($MgSO_4$), and concentrated to a residue that was triturated in hot 2-propanol. After storage at room temperature for 30 min, the solid was collected to give **29** (13.5 mg, 13%): mp 244−248 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.3 (s, 1 H, NH), 10.4 (s, 1 H, NH), 9.06 (s, 1 H), 9.02 (s, 1 H), 8.67 (s, 1 H), 8.64 (t, J = 5.8 Hz, 1 H, NH), 8.17 (d, J = 1.7 Hz, 1 H), 7.88 (dt, J = 7.5, 1.9, 1.9 Hz, 1 H), 7.65 (s, 1 H, imidazole H), 7.37 (m, 2 H), 7.29 (d, J = 14.9 Hz, 1 H, olefinic), 7.20 (t, J = 1.2 Hz, 1 H, imidazole H), 7.08 (d, J = 14.9 Hz, 1 H, olefinic), 6.90 (t, J = 1.2 Hz, 1 H, imidazole H), 4.01 (t, J = 7.0 Hz, 2 H, CH_2N), 3.14 (q, J = 6.8 Hz, 2 H, CH_2NH, coalesces to t with D_2O), 1.91 (quintet, J = 7.0 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{23}H_{21}BrN_8O_2 \cdot 0.75H_2O$) C, H, N.

(2*E*)-*N*^{1}-[4-(3-Chloro-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-*N*^{4}-[3-(4-morpholinyl)propyl]-2-butenediamide (31). 6-Amino-4-(3-chloro-4-fluoroanilino)pyrido[3,4-*d*]-pyrimidine[14] (**48**) (1.3 g, 4.5 mmol) and **47a** (4.36 g, 18 mmol) in dry pyridine (22.5 mL) were dissolved by gentle heating, then the solution was cooled in an ice bath under N_2 and treated with pulverized EDCI·HCl (4.31 g, 22.5 mmol). The reaction was stirred at 0−5 °C for 4 h, then poured onto a mixture of EtOAc/5% aqueous $NaHCO_3$. The precipitated solid was collected, washed with water, then dissolved in hot MeOH. The solution was treated with limited conc HCl and set aside to crystallize, giving **31** (1.34 g, 51%) as a partial HCl salt: mp 232−235 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.3 (s, 1 H, NH), 10.5 (br s, ∼1 H, R_2N^+H), 10.4 (s, 1 H, NH), 9.06 (s, 1 H), 9.01 (s, 1 H), 8.79 (br s, 1 H, CH_2NH), 8.66 (s, 1 H), 8.14 (dd, J = 2.7 Hz, J_{H-F} = 7.0 Hz, 1 H), 7.83 (m, 1 H), 7.48 (dd, J = 9.0 Hz, J_{H-F} = 9.0 Hz, 1 H), 7.30 (d, J = 15.2 Hz, 1 H, olefinic), 7.20 (d, J = 15.2 Hz, 1 H, olefinic), 3.8 (br s, 4 H, morph. CH_2), 3.25 (q, J = 6.6 Hz, 2 H, CH_2NH, coalesces to t with D_2O), 3.0 (br s, 6 H, morph. CH_2, $NCH_2CH_2CH_2$), 1.85 (br s, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{24}H_{25}ClFN_7O_3 \cdot HCl \cdot 2H_2O$) C, H, N.

(2*E*)-*N*^{1}-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-*N*^{4}-[3-(dimethylamino)propyl]-*N*^{1}-methyl-2-butenediamide (30). Reaction of **37** (132 mg, 0.4 mmol) and **47b** (380 mg, 1.6 mmol) with EDCI·HCl (392 mg) in pyridine (2 mL) as above gave a crude product that was extracted with EtOAc (2×) to remove organic impurities. The combined EtOAc extracts were washed with water, then all aqueous phases were combined and adjusted to pH 9 with 2% aqueous NaOH. The resulting precipitate was collected, washed well with water, and then triturated in 2-propanol at 0 °C for 30 min to give **30** (64 mg, 31%): mp 228−230 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.1 (s, 1 H, NH), 9.15 (s, 1 H), 8.81 (s, 1 H), 8.47 (s, 1 H), 8.43 (t, J = 5.5 Hz, 1 H, CH_2NH), 8.20 (s, 1 H), 7.90 (d, J = 7.2 Hz, 1 H), 7.39 (m, 2 H), 6.95 (d, J = 14.9 Hz, 1 H, olefinic), 6.72 (d, J = 14.9 Hz, 1 H, olefinic), 3.47 (s, 3 H, $CONCH_3$), 3.09 (q, J = 6.0 Hz, 2 H, CH_2NH, coalesces to t with D_2O), 2.15 (t, J = 7.2 Hz, 2 H, $Me_2NCH_2CH_2CH_2$), 2.06 (s, 6 H, $N(CH_3)_2$), 1.50 (quintet, J = 7.2 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{23}H_{26}BrN_7O_2 \cdot 0.25H_2O$) C, H, N.

***N*-[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]ethylenesulfonamide (32): Example of Method of Scheme 1.** To a stirred solution of **38** (0.25 g, 0.82 mmol) in THF (20 mL) under nitrogen was added Et_3N (230 μL), a catalytic amount of DMAP and chloroethanesulfonyl chloride (120 μL, 1.15 mmol) dropwise. The reaction was stirred at room temperature for 1 h and then diluted with saturated $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and chromatographed on silica gel eluting with MeOH/CH_2Cl_2/EtOAc (2:48:50), then recrystalized from CH_2Cl_2/hexane to give **32** (53 mg, 16%): mp 261−265

°C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.02 (s, 1 H, SO_2NH), 10.25 (s, 1 H, NH), 9.02 (s, 1 H), 8.67 (s, 1 H), 8.15 (br s, 1 H, H-2′), 8.00 (s, 1 H), 7.87 (dt, J = 7.2, 1.9, 1.9 Hz, 1 H, H-6′), 7.40 (partially obsc. t, J = 7.9 Hz, 1 H, H-5′), 7.37 (partially obsc. dt, J = 7.8, 1.9, 1.9 Hz, 1 H, H-4′), 7.07 (dd, J = 16.5, 9.9 Hz, 1 H, CH_2CHSO_2), 6.30 (d, J = 16.5 Hz, 1 H, CH_2CHSO_2), 6.09 (d, J = 9.9 Hz, 1 H, CH_2CHSO_2). Anal. ($C_{15}H_{12}BrN_5O_2S\cdot 0.25H_2O$) C, H, N.

***N*-[4-(3-Bromoanilino)-6-quinazolinyl]ethylenesulfonamide (33).** To a stirred solution of **39** (0.30 g, 0.95 mmol) in THF (20 mL) under nitrogen were added Et_3N (3.5 mol equiv, 3.33 mmol, 245 μL), a catalytic amount of DMAP and chloroethanesulfonyl chloride (1.2 mol equiv, 1.14 mmol, 119 μL) dropwise. Workup as above followed by chromatography on silica gel eluting with MeOH/CH_2Cl_2/EtOAc (3:47:50) and crystallization from CH_2Cl_2/hexane gave **33** (210 mg, 54%): mp 217 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.31 (s, 1 H, SO_2NH), 9.96 (s, 1 H, NH), 8.60 (s, 1 H, H-2), 8.20 (d, J = 2.0 Hz, 1 H, H-5), 8.14 (br s, 1 H, H-2′), 7.85 (br d, J = 7.9 Hz, 1 H, H-6′), 7.81 (d, J = 8.9 Hz, 1 H, H-8), 7.67 (dd, J = 8.9, 2.1 Hz, 1 H, H-7), 7.37 (t, J = 8.0 Hz, 1 H, H-5′), 7.32 (br d, J = 8.1 Hz, 1 H, H-4′), 6.90 (dd, J = 16.4, 9.8 Hz, 1 H, CH_2CHSO_2), 6.17 (d, J = 16.4 Hz, 1 H, CH_2CHSO_2), 6.06 (d, J = 9.8 Hz, 1 H, CH_2CHSO_2). Anal. ($C_{16}H_{13}BrN_4O_2S$) C, H, N.

2-{[4-(3-Bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]sulfonyl}ethanol (36): Example of Method of Scheme 6. A nitrogen-purged solution of 2-mercaptoethanol (1.75 mL, 25 mmol) and 4-(3-bromoanilino)-6-fluoropyrido[3,4-*d*]pyrimidine[17] (**49**) (1.6 g, 5 mmol) in DMSO (10 mL) was treated with anhydrous cesium carbonate (3.26 g, 10 mmol). The stirred solution was heated at 50 °C for 2 h, then poured into 2% aqueous HCl (180 mL). After stirring for 15 min, the solids were collected, washed well with water, and dissolved in DMF. The solution was poured into EtOAc/water (1:1) the resulting mixture was extracted with EtOAc (3×). The combined extracts were washed with brine, dried ($MgSO_4$) and filtered through a column of silica gel. The filtrate was concentrated to a solid that was triturated in EtOAc to give 2-{[4-(3-bromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]sulfanyl}ethanol (**50**) (1.24 g, 66%): mp 182−185 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.03 (s, 1 H, NH), 9.10 (s, 1 H), 8.69 (s, 1 H), 8.35 (s, 1 H), 8.22 (t, J = 1.9 Hz, 1 H), 7.91 (dt, J = 7.7, 1.9 Hz, 1 H), 7.42−7.34 (m, 2 H), 5.04 (t, J = 5.5 Hz, 1 H, OH), 3.68 (dd, J = 6.8, 5.7 Hz, 2 H), 3.36 (t, J = 6.8 Hz, 2 H); MS (APCI) *m*/*z* (relative %) 374.8 (49), 375.8 (10), 376.9 (100), 377.8 (23), 378.9 (63), 379.8 (14). Anal. ($C_{15}H_{13}BrN_4OS$) C, H, N.

A stirred suspension **50** (755 mg, 2 mmol) in $CHCl_3$ (30 mL) at 0−5 °C was treated with MCPBA (1.27 g of 57−86%) and slowly warmed to 25 °C over 4 h. After 14.5 and 17.5 h further MCPBA (720 mg and 720 mg) was added. After 19.5 h total reaction time, the suspension was cooled to 0−5 °C, treated with DMSO (2 mL) and allowed to warm to 20 °C for 30 min. The mixture was then partitioned between EtOAc and 5% aqueous $NaHCO_3$. The organic phase was washed with brine, dried ($MgSO_4$), concentrated to small volume and flash chromatographed on silica gel, eluting with EtOAc to give **34** (540 mg, 66%): mp (EtOAc) 210−212 °C; ^{1}H NMR (CF_3CO_2H) δ 10.96 (s, 1 H), 10.90 (s, 1 H),) 10.42 (s, 1 H), 9.47 (s, 1H), 9.16 (d, J = 8.2 Hz, 1 H), 9.05 (d, J = 8.2 Hz, 1 H), 8.83 (t, J = 8.0 Hz, 1 H), 5.81(t, J = 5.2 Hz, 2 H), 5.43 (t, J = 5.2 Hz, 2 H); MS (APCI) *m*/*z* (relative %) 378.7 (39), 380.7 (45), 408.7 (100), 409.7 (15), 410.7 (97), 411.7 (17). Anal. ($C_{15}H_{13}BrN_4O_3S$) C, H, N.

***N*-(3-Bromophenyl)-6-(vinylsulfonyl)pyrido[3,4-*d*]pyrimidin-4-amine (35).** Methanesulfonyl chloride (9.3 μL, 0.12 mmol) was added dropwise to a stirred suspension of **34** (41 mg, 0.1 mmol) and Et_3N (31 μL, 0.22 mmol) in CH_2Cl_2 (0.5 mL) under N_2 at 0−5 °C. Additional charges of methanesulfonyl chloride (9.3 μL) were added after 45 min and 1.5 h, the latter with additional Et_3N (50 μL). After a total of 2.5 h the cold solution was quenched with 5% aqueous $NaHCO_3$, then extracted with EtOAc (2×). The combined organic extracts were dried ($MgSO_4$) then filtered through a pad of flash silica gel to give **35** (17 mg, 44%): mp (EtOAc) 214−217 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.64 (s, 1 H, NH), 9.30 (s, 1 H), 9.25 (s, 1 H), 8.87 (s, 1 H), 8.16 (s, 1 H), 7.89−7.85 (m, 1 H), 7.39−7.33 (m, 2 H), 7.17 (dd, J = 10.0, 16.5 Hz, 1 H), 6.46 (d, J = 16.4 Hz, 1 H), 6.37 (d, J = 10.0 Hz, 1 H). Anal. ($C_{15}H_{11}BrN_4O_2S\cdot 0.25H_2O$) C, H, N.

***N*-(3-Bromophenyl)-6-(vinylsulfinyl)pyrido[3,4-*d*]pyrimidin-4-amine (36): Example of Method of Scheme 6.** A suspension of **50** (226 mg, 0.6 mmol) and 3-phenyl-2-(phenylsulfonyl)oxaziridine (180 mg) in $CHCl_3$ (6 mL) was stirred at room temperature for 3 h. The mixture was diluted with *tert*-butyl methyl ether (6 mL) and the precipitate was collected to give 2-[4-(3-bromophenylamino)pyrido[3,4-*d*]pyrimidine-6-sulfinyl]ethanol (**51**) (210 mg, 89%): mp 233−235 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.6 (s, 1 H, NH), 9.25 (s, 1 H), 9.04 (s, 1 H), 8.86 (s, 1 H), 8.23 (d, J = 1.9 Hz, 1 H), 7.95 (dt, J = 2.2, 2.2, 7.2 Hz, 1 H), 7.39 (m, 2 H), 5.09 (dd, J = 5.1, 5.8 Hz, 1 H, OH), 3.87 (m, 1 H, $HOCH_2CH_2$), 3.80 (m, 1 H, $HOCH_2CH_2$), 3.32 (m, 1 H, $HOCH_2CH_2$), 2.98 (dt, J = 4.3, 4.3, 13.3 Hz, 1 H, $HOCH_2CH_2$), 1.91 (dd, J = 7.0, 1.4 Hz, 3 H). Anal. ($C_{15}H_{13}BrN_4O_2S$) C, H, N.

Methanesulfonyl chloride (0.056 mL, 0.72 mmol) was added to an ice-cold suspension of **51** (117 mg, 0.3 mmol) and Hunig's base (0.3 mL, 1.7 mmol) in dichloroethane (3 mL). After 1 h, the mixture was brought to room temperature and maintained there for 30 min. DBU (316 mg, 2 mmol) was added, and the solution stirred for 30 min, then quenched with water. The mixture was extracted with dichloroethane (2×) and the combined extracts were washed with 2% aqueous HCl, dried (Mg_2SO_4), and filtered through a pad of flash silica gel, eluting with EtOAc. Concentration of the combined product eluates and trituration of the resulting solid with MeOH gave **36** (69 mg, 61%): mp 213-214 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.61 (s, 1 H, NH), 9.25 (s, 1 H), 9.00 (s, 1 H), 8.86 (s, 1 H), 8.22 (s, 1 H), 7.93 (d, J = 7.0 Hz, 1 H), 7.38 (m, 2 H), 7.12 (dd, J = 9.6, 16.4 Hz, 1 H), 6.16 (d, J = 16.4 Hz, 1 H), 6.06 (d, J = 9.6 Hz, 1 H). Anal. ($C_{15}H_{11}BrN_4OS$) C, H, N.

Tyrosine Kinase Assays. EGFR tyrosine kinase was purified as described previously.[21] Enzyme assays for $IC_{50[app]}$ determinations were performed in 96-well filter plates (Millipore MADVN6550, Millipore, Bedford, MA). The total volume was 0.1 mL containing 20 mM Hepes, pH 7.4, 50 mM sodium vanadate, 40 mM magnesium chloride, 10 μM adenosine triphosphate (ATP) containing 0.5 mCi of [^{32}P]ATP, 20 mg of polyglutamic acid/tyrosine (Sigma Chemical Co., St. Louis, MO), 10 ng of EGFR tyrosine kinase and appropriate dilutions of inhibitor. All components except the ATP are 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 and the plate incubated at 25 °C for 10 min. The reaction was terminated by addition of 0.1 mL of 20% trichloroacetic acid (TCA). The plate was kept at 4 °C for at least 15 min to allow the substrate to precipitate. The wells was then washed 5 times with 0.2 mL of 10% TCA and ^{32}P incorporation determined with a Wallac beta plate counter (Wallac, Inc., Gaithersburg, PA).

Irreversibility Test Protocol. A431 human epidermoid carcinoma cells were grown in 6-well plates to about 80% confluency and then incubated in serum-free media for 18 h. Duplicate sets of cells were treated with 2 mM of designated compound to be tested as an irreversible inhibitor for 2 h. One set of cells was then stimulated with 100 ng/mL of EGF for 5 min and extracts made as described under the Western blotting procedure. The other set of cells was washed free of the compound with warmed serum-free media, incubated for 2 h, washed again, incubated another 2 h, washed again, and then incubated a further 4 h. This set of cells was then stimulated with EGF and extracts made similar to the first set of cells.

In Vivo Chemotherapy. Evaluation of in vivo anticancer effectiveness was performed as described previously.[15] The A431 epidermoid, H125 non-small-cell lung, and MCF-7 breast xenografts were maintained by serial in vivo passage in and anticancer effectiveness evaluated in nude mice (Charles River Breeding Laboratories). Compounds **7** and **31** were adminis-

tered as suspensions in 0.5% methylcellulose in water due to insufficient solubility at the desired dosage concentration. Both compounds were dosed orally in a fixed volume of 0.5 mL. Host body weight change data are reported as the maximum treatment-related weight loss in these studies. Calculation of tumor growth inhibition (% T/C) and tumor growth delay (T−C) was performed as described previously.[22−24]

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