Tyrosine Kinase Inhibitors. 15. 4-(Phenylamino)quinazoline and 4-(Phenylamino)pyrido[*d*]pyrimidine Acrylamides as Irreversible Inhibitors of the ATP Binding Site of the Epidermal Growth Factor Receptor

Jeff B. Smaill,[†] Brian D. Palmer,[†] Gordon W. Rewcastle,[†] William A. Denny,*[,†] Dennis J. McNamara,[‡] Ellen M. Dobrusin,[‡] Alexander J. Bridges,[‡] Hairong Zhou,[‡] H. D. Hollis Showalter,[‡] R. Thomas Winters,[‡] Wilbur R. Leopold,[‡] David W. Fry,[‡] James M. Nelson,[‡] Veronika Slintak,[‡] William L. Elliot,[‡] Billy J. Roberts,[‡] Patrick W. Vincent,[‡] and Sandra J. Patmore[‡]

Auckland Cancer Society Research Centre, 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

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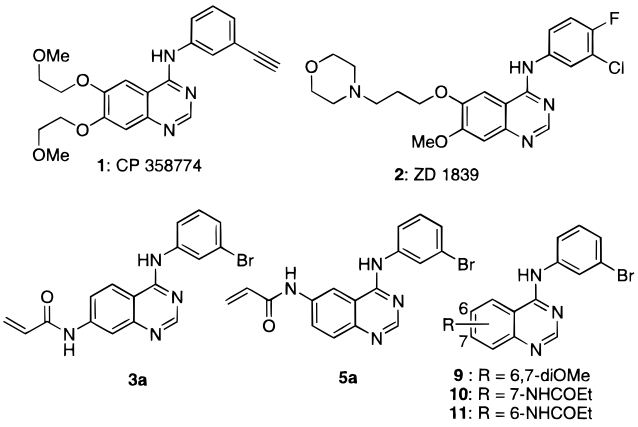
A series of 6- and 7-acrylamide derivatives of the 4-(phenylamino)quinazoline and -pyridopyrimidine classes of epidermal growth factor receptor (EGFR) inhibitors were prepared from the corresponding amino compounds by reaction with either acryloyl chloride/base or acrylic acid/1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. All of the 6-acrylamides, but only the parent quinazoline 7-acrylamide, were irreversible inhibitors of the isolated enzyme, confirming that the former are better-positioned, when bound to the enzyme, to react with the critical cysteine-773. Quinazoline, pyrido[3,4-*d*]pyrimidine, and pyrido[3,2-*d*]pyrimidine 6-acrylamides were all irreversible inhibitors and showed similar high potencies in the enzyme assay (likely due to titration of the available enzyme). However the pyrido[3,2-*d*]pyrimidine analogues were 2−6-fold less potent than the others in a cellular autophosphorylation assay for EGFR in A431 cells. The quinazolines were generally less potent overall toward inhibition of heregulin-stimulated autophosphorylation of *erb*B2 (in MDA-MB-453-cells), whereas the pyridopyrimidines were equipotent. Selected compounds were evaluated in A431 epidermoid and H125 non-small-cell lung cancer human tumor xenografts. The compounds showed better activity when given orally than intraperitoneally. All showed significant tumor growth inhibition (stasis) over a dose range. The poor aqueous solubility of the compounds was a drawback, requiring formulation as fine particulate emulsions.

Introduction

Following numerous reports that overexpression of the epidermal growth factor receptor (EGFR) is a marker for poor prognosis in a significant proportion of human tumors,[1,2] selective inhibitors of tyrosine phosphorylation by EGFR have become an important class of potential anticancer drugs.[3−5] Several classes of inhibitors have been reported, with the most potent and selective being the 4-(phenylamino)quinazolines[6−9] and related 4-(phenylamino)pyrido[*d*]pyrimidines.[10−12] Kinetic studies[7,12] show that these compounds selectively inhibit EGF-stimulated signal transduction by reversibly binding at the ATP site of EGFR. Many of these compounds are extremely potent inhibitors of the isolated enzyme and inhibit EGF-stimulated EGFR autophosphorylation in cells for up to 4 h.[8] Two 4-(phenylamino)quinazolines, CP 358774 (**1**) and ZD 1839 (**2**), are reported to be in clinical trial.[13,14]

Despite the high potency and prolonged inhibition of EGFR function reported for some of these reversible inhibitors, the high intracellular concentrations of ATP make it difficult to reach sufficiently high concentrations in vivo to fully shut down EGF-stimulated signal transduction for long periods. We[15] and others[16] have therefore been studying the development of irreversible inhibitors based on the 4-(phenylamino)quinazolines. We recently reported[15] that the acrylamides (**3a** and **5a)** are potent, selective, irreversible inhibitors of the EGFR tyrosine kinase, with improved in vivo antitumor activity compared to the related reversible analogues **10** and **11**.



These molecules were designed initially to trap the Cys-773 of EGFR, which had been identified from sequence homology data as being very uncommon at that position in kinases but shared by EGFR, *erb*B-2, and *erb*B-4. Sequence homology suggested that it is the EGFR equivalent of Asn-127 in PKA.[17] As this residue

[†] The University of Auckland.
[‡] Parke-Davis Pharmaceutical Research.

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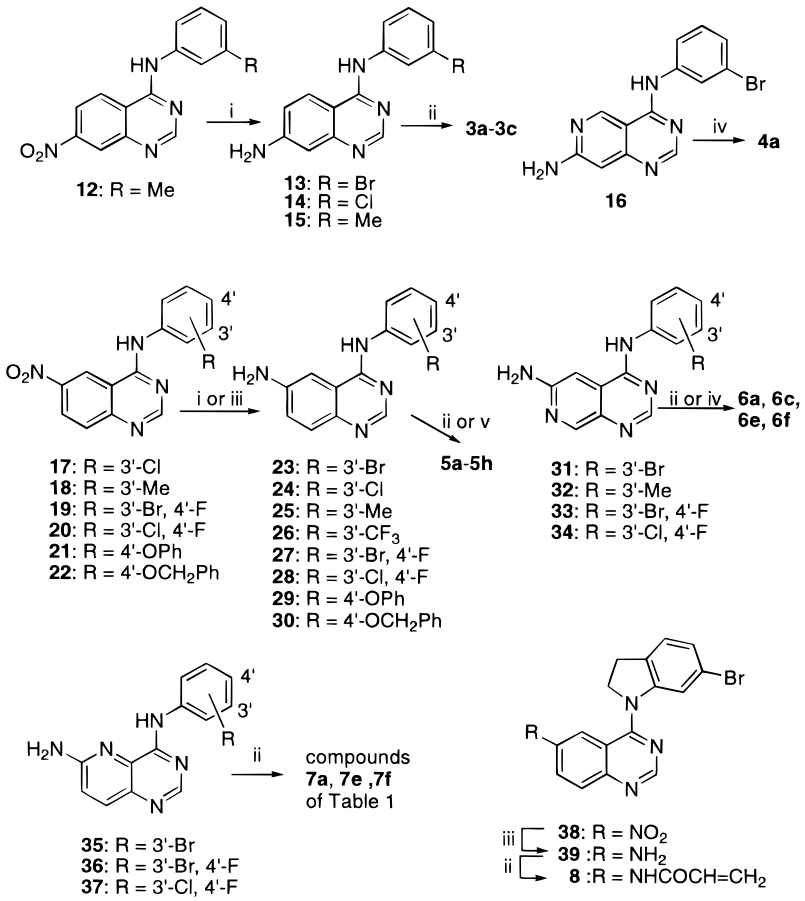
Scheme 1[a]



[a] (i) Raney Ni/H_2/MeOH/25 °C/15 h; (ii) acrylic acid/EDCI·HCl/DMF (and/or THF, DMA)/Et_3N or pyridine/25 °C/20 h; (iii) Fe/AcOH/H_2O/EtOH/reflux/1 h; (iv) acryloyl chloride/THF:DMF/DMAP/Et_3N/0−20 °C/20 h; (v) acrylic acid/isobutyl chloroformate/THF/DMF/Et_3N/0 °C/5 h.

has been shown by X-ray crystallographic studies to H-bond to the 2′-hydroxyl of the ATP ribose, it should be in relatively close proximity to the hydrophobic adenine binding pocket where we presumed that the bicyclopyrimidine binds. The ability to trap this cysteine was confirmed by the demonstration that 2-thioadenosine is an irreversible inhibitor of EGFR, but not of PDGFR, where the equivalent residue is not a cysteine.[17] Our initial modeling studies of the binding of reversible anilinoquinazoline and anilinopyridopyrimidine inhibitors[15,18] suggested a binding mode whereby the 6- and 7-positions of the bicyclic chromophore point out of the ATP binding pocket toward the solvent. This explains why both positions can be used to add bulky solubilizing functionalities to inhibitors without losing binding affinity.[13,14,19,20] When an acrylamido side chain was attached to the 6-position in the model, the γ-carbon was placed only 3.5 Å from the cysteinyl sulfur, whereas at the 7-position the corresponding distance was nearly 8 Å.[15] These distances suggest that the 6-acrylamido side chain is optimally placed for reaction with Cys-773 in its most stable binding mode, whereas the 7-acrylamido side chain would require some movement in the binding site to allow it to approach close enough to the sulfur for Michael addition to occur. This is consistent with the kinetics of alkylation,[15] which show extremely rapid alkylation by the 6-acrylamide and much slower alkylation by the 7-acrylamide.

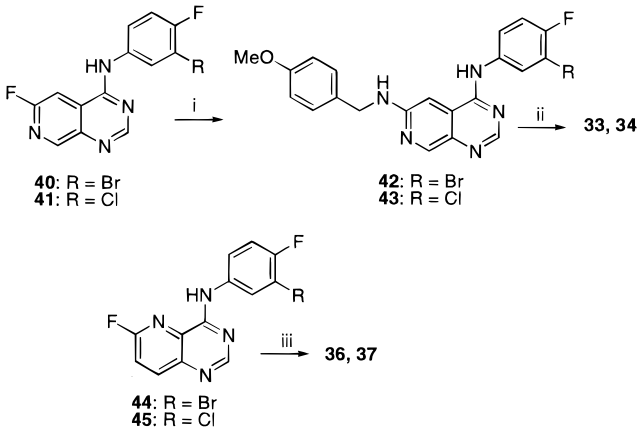
We now report the synthesis and biological activity of a range of 6- and 7-substituted acrylamidoquinazolines and acrylamidopyrido[*d*]pyrimidines, made to further investigate the structure−activity requirements for irreversible inhibition of EGFR and to provide potentially improved analogues in terms of potency against EGFR and *erb*B2, solubility, and in vivo antitumor activity.

Chemistry

The 6- and 7-acrylamides **3**−**8** of Table 1 were prepared from the corresponding 7-aminoquinazolines **13**−**15**, 6-aminoquinazolines **23**−**30**, 7-aminopyrido[4,3-*d*]pyrimidine **16**, 6-aminopyrido[3,4-*d*]pyrimidines **31**−**34**, and 6-aminopyrido[3,2-*d*]pyrimidines **35**−**37** usually by reaction either with acryloyl chloride and a base (pyridine or triethylamine) or with acrylic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) (Scheme 1).

For the synthesis of **5c**, the mixed anhydride from acrylic acid and isobutyl chloroformate was used. Many of the required amino compounds were known,[8,10,11,21] and new ones were prepared by similar methods (Schemes 1 and 2). Yields for the final acylation step were in the range 20−60%, with varying amounts of polymeric material also being formed. The pyridopyrimidines were less reactive to amide formation than the corresponding quinazolines.

Scheme 2[a]



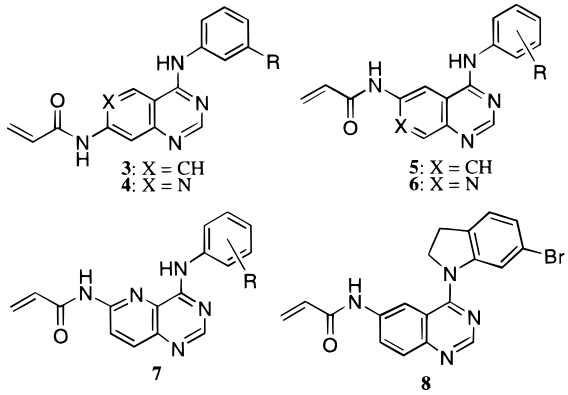
[a] (i) 4-OMePhCH$_2$NH$_2$/DMSO/100 °C/16 h; (ii) TFA/reflux/30 min; (iii) NH$_3$(g)/EtOH or *i*-PrOH/110 °C/18 h (pressure).

Results and Discussion

The structures and physicochemical properties of the acrylamides studied are listed in Table 1, together with their potencies for inhibition of phosphorylation of a glutamic acid/tyrosine random copolymer substrate by isolated EGFR enzyme, inhibition of EGF-stimulated autophosphorylation of EGFR in A431 cells, and inhibition of heregulin-stimulated autophosphorylation of *erb*B2 in MDA-MB-453 cells.[12] The nature of the inhibition of the EGFR enzyme was also determined for each compound, but not by rigorous biochemical means as this requires radiolabeling and mass spectroscopic methods. Instead, a variation of the cellular autophosphorylation assay of A431 cells was used. The cells were incubated with a fixed concentration (2 μM) of the inhibitor for 1 h and then washed free of drug. After 8 h EGF was added; after a further 10-min incubation the cells were lysed, and the degree of inhibition of autophosphorylation was measured. With this protocol, even the very potent (IC_{50} 29 pM) reversible inhibitor **9** showed only 5% inhibition after 8 h, whereas the verified[15] irreversible inhibitors **3a** and **5a** showed 89% and 100% inhibition, respectively.

Compounds which showed 80% or greater inhibition after the 8-h washout were designated as irreversible inhibitors. Those which showed 20−80% inhibition were designated as partially irreversible under the test conditions (although in reality they can almost certainly fully inactivate the enzyme via alkylation given sufficient time). Those which produced less than 20% inhibition were classified as reversible. If alkylation is slow relative to the length of time of the incubation, not all of the enzyme may be alkylated. Also, provided unreacted inhibitor does not remain in the cells to alkylate newly synthesized receptor (the half-life for turnover of EGFR in unstimulated A431 cells is ∼20 h), some activity would be expected after the washout period from newly synthesized receptor. Conversely, if a reversible inhibitor were unusually well-sequestered in cells, as has been demonstrated previously for some reversible quinazolines,[8] it could show up as a false positive in this assay. Thus the fully reversible Zeneca clinical candidate **2**, which is strongly sequestered in cells, produced 100% inhibition after the 8-h washout period, demonstrating that the above method can produce false positives for irreversible inhibition.

Table 1. Physicochemical and EGFR Inhibitory Properties of 4-(Phenylamino)quinazoline and 4-(Phenylamino)pyrido[*d*]pyrimidine Acrylamides



			isolated enzyme $IC_{50[app]}$ (nM)	autophosphorylation IC_{50} (nM)		
no.	R	mp (°C)	EGFR[a]	EGFR (A431)[b]	HER (MDA)[c]	irrev[d]
3a	Br	>265	0.45	14	73	yes
3b	Cl	296.5−298.5	0.25	53		part
3c	Me	269.5−270	1.6	90		no
4a	Br	215−220	0.54	108		part
5a	3′-Br	258−261	0.70	4.3	5.7	yes
5b	3′-Cl	223−227	1.6	2.3		yes
5c	3′-Me	247−248	0.42	4.7	22	yes
5d	3′-CF_3	195−199	0.91	4.5		yes
5e	3′-Br, 4′-F	276−278.5	0.69	2.7	7.3	yes
5f	3′-Cl, 4′-F	260−262	0.75	3.1	4.3	yes
5g	4′-OPh	185−191	5.8	4.8	8.6	yes
5h	4′-OBn	227−229	3.6	4.2	7.6	yes
6a	3′-Br	243−245	0.91	3.4	3.9	yes
6c	3′-Me	221−223	0.48	8.3	8.8	yes
6e	3′-Br, 4′-F	245−248	0.72	2.1	1.1	yes
6f	3′-Cl, 4′-F	>245 dec	0.77	3.3	3.0	yes
7a	3′-Br	226−228	1.1	5.6		yes
7e	3′-Br, 4′-F	241−243	1.2	18	26	yes
7f	3′-Cl, 4′-F	247	0.75	18	12	yes
8		214−216	0.40	9.9		yes

[a] $IC_{50[app]}$ (nM) to inhibit the phosphorylation of a poly(glutamic acid)/tyrosine random copolymer by EGFR (prepared from human A431 carcinoma cell vesicles by immunoaffinity chromatography). See Experimental Section for details. Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [b,c] IC_{50} values (nM) for inhibition of autophosphorylation of EGFR (in A431 cells) or *erb*B2 (in MDA-MB-453 cells) in culture. Values are the average of two experiments; see Experimental Section for details. [d] Irreversible inhibition is defined as no detection of phosphorylated EGFR in A431 cells 8 h after washing cells free of the inhibitor.

The irreversibility assay should thus be viewed as a method for distinguishing whether a particular chemical modification permits or destroys irreversibility. Compounds categorized as "partially irreversible" are likely capable of irreversibly inhibiting the enzyme, but at such a rate that the process is not complete within the 1-h incubation period. Because we believe that truly erroneous results are rare, and because slow "partial" alkylators proved no more active in vivo than ones which show up as fully reversible, the assay is of value as a high-throughput initial classification of candidate compounds.

The influence of the positioning of the acrylamide, aza atom substitution in the quinazoline chromophore, and (in 6-acrylamidoquinazolines) the effect of substituents in the aniline ring on both irreversibility and inhibitory potency were evaluated. In these evaluations, it must be borne in mind that equilibrium enzyme kinetics are

not valid for irreversible compounds and that IC_{50} values for both the isolated enzyme and cellular data depend on the rate and completeness of alkylation (which in turn depend on the reversible binding affinity of each acrylamide-bearing template) as well as on the concentration of enzyme present. In fact, for irreversible inhibitors 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 ($IC_{50[app]}$). The concentration of EGFR in the isolated enzyme assays is calculated at 1.18 nM and was held as constant as possible (<10% variation). All isolated enzyme assays were carried out identically over a 10-min incubation period and used the same enzyme preparation. The data in Table 1 are thus not kinetic parameters, but comparative data for each compound under precisely defined conditions. For rapid alkylators, where the addition reaction goes to completion within the incubation period, the $IC_{50[app]}$ values should all be similar (approximately one-half the concentration of the enzyme). However, it is still possible to obtain low-nanomolar $IC_{50[app]}$ values in the isolated enzyme assay for partially irreversible (slow alkylators) and reversible inhibitors due to their intrinsically high reversible binding affinity.

Positioning of the Acrylamide Michael Acceptor. We have previously reported,[15] from studies with **3a** and **5a**, that a 6-acrylamide appears better positioned than a 7-acrylamide (4 Å closer to the Cys-773 thiol) to form the required bond. The present work provides additional support for this proposal, by a comparison of the properties of two more pairs of 6- and 7-acrylamides (**3b** and **5b**, **3c** and **5c**). All three 6-acrylamides (**5a**−**c**) are completely irreversible inhibitors of autophosphorylation in A431 cells (Table 1) and have isolated enzyme $IC_{50[app]}$ values close to those expected for compounds capable of rapid and complete alkylation of the enzyme (see above). In contrast, only the original 3′-Br analogue **3a** in the 7-acrylamide series proved irreversible in the 8-h washout assay. The 3′-Cl and 3′-Me analogues **3b** and **3c** were partial irreversible and reversible inhibitors, respectively. Although the $IC_{50[app]}$ values of these two sets of compounds against the isolated enzyme were not very different, the fully irreversible 6-acrylamides were much more potent inhibitors than the partially irreversible 7-acrylamides in the cellular autophosphorylation assays. These results suggest that while high potency in the isolated enzyme assay is desirable, it does not necessarily distinguish between compounds capable of rapid and complete alkylation of the enzyme and compounds capable of potent enzyme inhibition via high-affinity reversible binding. However there appears to be a correlation between irreversible binding (as determined by the 8-h washout assay) and potency in the cellular autophosphorylation assays, suggesting that this assay may be more useful in ranking compounds for advanced evaluation and development. These results are consistent with the idea that the ATP binding pocket does not have enough flexibility to allow an optimal distance between the 7-acrylamide and Cys-773 and that as the intrinsic binding to the enzyme decreases (as seen from the SAR for reversible inhibition) when going from 3′-Br to 3′-Cl to 3′-Me, alkylation becomes slow enough on the time scale of the experiment to become unimportant.

Aza Substitution in the Quinazoline Chromophore. Five sets of compounds were prepared where the position of the acrylamide and the nature of the aniline substituents were unchanged (within a particular set), while the central chromophore was varied from quinazoline to pyrido[3,4-*d*]pyrimidine, pyrido[3,2-*d*]-pyrimidine, and pyrido[4,3-*d*]pyrimidine (Table 1). We have previously shown[11] that all of these pyrido[*d*]-pyrimidine chromophores bind reversibly at the ATP site of EGFR.

In the 7-acrylamide set (**3a** and **4a**), changing the quinazoline to pyrido[4,3-*d*]pyrimidine resulted in loss of irreversible inhibition and a corresponding loss in potency in the autophosphorylation assay (although retaining comparable $IC_{50[app]}$ values against isolated EGFR). This reinforces the above view that the 7-acrylamide is not well-placed to react with Cys-773, especially as the aza substituent would be expected to increase the reactivity of the acrylamide as a Michael acceptor. The remaining sets were 6-acrylamide analogues that all retained irreversible inhibition of EGFR, indicating a broad tolerance for structural variation when the acrylamide is optimally situated. Compounds **5c** and **6c**, which compare quinazoline and pyrido[3,4-*d*]pyrimidine analogues with a 3′-Me aniline substituent, had similar $IC_{50[app]}$ values against the isolated enzyme (close to the maximum expected), consistent with rapid, irreversible inhibition. The remaining three sets of three (**5a**, **6a**, **7a**; **5e**, **6e**, **7e**; and **5f**, **6f**, **7f**) compare quinazoline, pyrido[3,4-*d*]pyrimidine, and pyrido[3,2-*d*]pyrimidine chromophores with 3′-bromo, 3′-bromo-4′-fluoro, and 3′-chloro-4′-fluoro aniline substituents, respectively. Again, $IC_{50[app]}$ values for inhibition of isolated enzyme were similar for all three chromophores and consistent with rapid irreversible inhibition.

However, the latter two pyrido[3,2-*d*]pyrimidines were somewhat less potent than the other analogues in the EGFR autophosphorylation assay. One possible explanation for this is that the aza substituent increases the electron deficiency of the Michael acceptor, making it too reactive to retain selectivity and allowing loss by indiscriminate alkylation. The aniline side chain is also known to have a different favored conformation when a 5-aza atom is present.[22] The quinazolines were generally slightly less potent inhibitors of *erb*B2 than EGFR in the cellular autophosphorylation assays, but the pyridopyrimidines proved equipotent in these assays. The latter compounds thus form a group of novel and very potent generic inhibitors of the *erb*B family of receptor TKs. We have previously reported[15] that ^{14}C-labeled **3a** binds covalently to both EGFR and *erb*B2, and it is thus very likely that the excellent activity shown by the pyridopyrimidines against *erb*B2 is largely due also to irreversible inhibition of this enzyme. Among reversible compounds, the pyridopyrimidines are more active than the corresponding quinazolines against *erb*B2 autophosphorylation.[23]

Variations in Side Chain Substituents. A series of 6-acrylamidoquinazolines (**5a**−**5h**) with variations in the aniline were investigated. The 3′-substituents were known from previous work to assist binding by interac-

Table 2. In Vivo Anticancer Activity of Selected 4-(Phenylamino)quinazoline and 4-(Phenylamino)pyrido[*d*]pyrimidine Acrylamides against Tumor Xenografts in Nude Mice

no.	tumor[a]	dose (mg/kg)[b]	schedule[c]	weight change (g)	T/C (%) on last therapy day[d]	T−C (days)[e]	net cell kill (log10)[f]
5a	A431	47	ip, b.i.d. days 7−11, 14−18, 21−25	−1.0	2	29.0	+0.6
	A431	150 (hdt)	po, b.i.d. days 10−24	−1.7	2	28.6	+1.0
	H125	50 (hdt)	ip, b.i.d. days 11−15, 18−22, 25−29	−1.5	15	13.9	−0.2
	H125	50 (hdt)	po, days 21−35	+	8	15.7	+0.1
5c	A431	75 (hdt)	ip, b.i.d. days 14−28	−2.2	11	32.3	+0.9
	A431	260 (hdt)	po, days 10−23	−1.3	3	28.2	+0.8
	H125	250 (hdt)	po, days 21−35	−0.1	24	10.5	−0.2
	H125	50 (hdt)	ip, b.i.d. days 11−15, 18−22, 25−29	0	15	11.9	−0.3
5f	A431	40 (hdt)	po, days 10−23	0	11	20.8	+0.4
	H125	200 (hdt)	po, days 21−35	−0.3	16	27.4	+0.9
6a	A431	12 (ldt)	ip, b.i.d. days 13−26	−2.4 (2/6)[g]	17	12.0	
	A431	200 (hdt)	po, days 10−24	+	11	24.3	+0.9
	H125	9.6	ip, b.i.d. days 11−15, 18−22, 25−29	−1.3	26	8.7	−0.5
	H125	200 (hdt)	po, days 21−35	+	20	16.5	+0.1
6e	A431	200 (hdt)	po, days 10−24	+	13	51.1	+1.7
	H125	200 (hdt)	po, days 15−29	−1.8	19	18.2	+0.2
6f	A431	200 (hdt)	po, days 10−24	−0.3	12	18.3	+0.4
	H125	200 (hdt)	po, days 15−29	−1.8	27	12.7	−0.1

[a] The indicated tumor fragments were implanted sc into the right axilla of mice on day 0. [b] hdt, highest dose tested; ldt, lowest dose tested. [c] 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, unless otherwise noted. [d] Ratio of median treated tumor mass/median control tumor mass × 100%. [e] The difference in days for the treated (T) and the control (C) tumors to reach 750 mg. [f] The net reduction in tumor burden, in logs, between the first and last treatments. [g] 2/6, 2 toxic deaths in a group of 6 animals.

tion with a lipophilic pocket in the ATP binding domain, while a 4′-F group has been shown[24] to extend plasma half-life by slowing down metabolism in the dianilinophthalimide EGFR inhibitors. All except the last two (**5g** and **5h**), with the more bulky side chains (4′-OPh and 4′-OBn), had similar $IC_{50[app]}$ values against the isolated enzyme (∼0.8 nM), consistent with irreversible inhibition. These showed lower potency against the isolated enzyme ($IC_{50[app]}$ = 5.8 and 3.6 nM, respectively), despite being fully irreversible in the 8-h washout assay. This suggests they have a lower binding affinity for EGFR than the other analogues (**5a**−**5f**) (presumably due to steric bulk in the 4′-position) and therefore a rate of alkylation that falls between the incubation times for the isolated enzyme and irreversibility assays (10 min and 1 h, respectively). These compounds also dramatically illustrate the apparent advantage of irreversible inhibition for cellular *erb*B2-stimulated autophosphorylation activity. The corresponding (reversible) and EGFR-selective[25] 6,7-dimethoxyquinazoline inhibitors have reported IC_{50} values of 19 and 11 nM against isolated EGFR, respectively, but IC_{50} values of 700 and 1200 nM in the *erb*B2 autophosphorylation assay.[26] Thus the EGFR (isolated enzyme) potencies are only increased about 3-fold, whereas the *erb*B2 (cellular) potencies are increased around 100-fold. The bromoindoline analogue **8**, a tethered aniline, was also fully irreversible and possessed potent isolated enzyme ($IC_{50[app]}$ = 0.40 nM) and cellular autophosphorylation activity.

In Vivo Studies. Compounds **5a**, **5c**, **5f**, **6a**, **6e**, and **6f** were evaluated in vivo against the A431 epidermoid and H125 non-small-cell lung cancer human tumor xenograft models. As rapid, irreversible inhibitors, all these compounds had essentially similar in vitro potencies (Table 1), so that any differences revealed in vivo might reflect differences in pharmacokinetics and metabolism. The tumor models were selected because they express at least two members of the *erb*B receptor family[20,27] and because of their responsiveness to reversible 4-(phenylamino)quinazoline inhibitors.[20] The compounds were dosed as suspensions, both ip and po. Ip dosing was generally more toxic, as evidenced by generally greater treatment-related weight loss, gross morphological liver changes, and attainment of maximum tolerated doses for **5a** and **6a**. With po dosing in particular, toxicity was not reached at the highest doses tested (Table 2), and no liver alterations were observed.

All of the compounds tested showed robust in vivo activity. On po dosing, all showed significant tumor growth inhibition over a dose range and activity at the highest doses tested without lethality or significant treatment-associated weight loss (>10%), indicating acceptable therapeutic indices. Net tumor cell kill values were around −0.5 to +0.5 log unit, indicating tumor stasis. Upon cessation of therapy treated tumor growth resumed, but in po dosing all of the compounds produced a growth delay at least as long as the dosing period against A431, and four of the six compounds did the same with the more refractory H125 tumors. However, it was not possible to discern major differences between the compounds, aside from the fact that ip dosing was apparently more toxic than po dosing.

Conclusions

The above results show that 6-acrylamides of 4-(phenylamino)quinazolines and -pyridopyrimidines are robust, irreversible inhibitors of the epidermal growth factor receptor, presumably by reaction at Cys-773, as previously shown[15] for the parent compound **5a**. Quinazoline, pyrido[3,4-*d*]pyrimidine, and pyrido[3,2-*d*]pyrimidine 6-acrylamides all showed irreversible inhibition and in general similar potencies in the isolated enzyme assay, suggesting titration of the available EGFR. The fact that the pyrido[3,2-*d*]pyrimidine analogues were somewhat less potent in the cellular autophosphorylation assays suggests another factor, possibly increased reactivity, altered absorption or metabolism. The quinazolines were generally less potent against *erb*B2 than EGFR in the cellular assays, but the pyridopyrimidines

were equipotent in both assays, providing an interesting class of generic inhibitors of the *erb*B family. The compounds showed encouraging in vivo activity, being cytostatic rather than cytotoxic, but with good therapeutic indices. Their poor aqueous solubility was a drawback, requiring formulation as fine particulate emulsions. While variation in aniline substitution improves solubility somewhat (in the order 3′-Br to 3′-Cl, 3′-CF_3, and 3′-CH_3), further substantial improvements are desirable, and this is the focus of continuing work.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or by Parke-Davis Pharmaceutical Research Analytical Department. 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 DRX-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 an a Finnigan MAT 900Q spectrometer. 2-Bromo-1-fluoro-4-nitrobenzene was prepared by bromination of 1-fluoro-4-nitrobenzene according to the literature procedure.[28,29] Iron dust reduction then gave 3-bromo-4-fluoroaniline[30] as an oil, which was used directly.

***N*-[4-((3-Bromophenyl)amino)quinazolin-7-yl]acrylamide (3a).** An ice-cold solution of 7-amino-4-[(3-bromophenyl)-amino]quinazoline[21] (**13**) (0.158 mg, 0.5 mmol) and acrylic acid (0.108 g, 1.5 mmol) in dry DMF (5 mL) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl; 0.288 g, 1.5 mmol). The mixture was stirred for 5 min at 0 °C, during which time it became homogeneous, and then at 20 °C for a further 3 h. The mixture was then poured into ice/water and basified with saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (3×), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The solid residue was dissolved in MeOH (100 mL) and filtered, and the filtrate was concentrated under reduced pressure to approximately 10 mL. The resulting precipitate was dried under vacuum at 80 °C to give **3a** (0.050 g, 28%): mp >265 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.61 (s, 1 H, CONH), 9.79 (s, 1 H, NH), 8.61 (s, 1 H, H-2), 8.50 (d, J = 8.9 Hz, 1 H, H-5), 8.26−8.23 (m, 2 H, H-2′, H-8), 7.91 (d, J = 8.0 Hz, 1 H, H-6′), 7.82 (dd, J = 9.2, 2.2 Hz, 1 H, H-6), 7.36 (t, J = 8.1 Hz, 1 H, H-5′), 7.30 (m, 1 H, H-4′), 6.51 (dd, J = 16.9, 10.1 Hz, 1 H, C*H*=CH_2), 6.36 (dd, J = 17.0, 1.9 Hz, 1 H, CH=CH_2), 5.86 (dd, J = 10.1, 1.9 Hz, 1 H, CH=CH_2). Anal. ($C_{17}H_{13}BrN_4O$) C, H, N.

***N*-[4-((3-Chlorophenyl)amino)quinazolin-7-yl]acrylamide (3b).** Similar reaction of 7-amino-4-[(3-chlorophenyl)-amino]quinazoline[8] (**14**) with acrylic acid and EDCI·HCl in DMF for 18 h gave a crude product that was dissolved in the minimum amount of MeOH at 25 °C and then concentrated under reduced pressure at 25 °C to one-sixth the volume. The resulting precipitate was recrystallized from MeOH below 0 °C to give **3b** (20%): mp 296.5−298.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.61 (br s, 1 H, CONH), 9.80 (s, 1 H, NH), 8.62 (s, 1 H, H-2), 8.50 (d, J = 9.0 Hz, 1 H, H-5), 8.25 (d, J = 2.0 Hz, 1 H, H-8), 8.13 (t, J = 2.0 Hz, 1 H, H-2′), 7.87−7.78 (m, 2 H, H-6,6′), 7.42 (t, J = 8.2 Hz, 1 H, H-5′), 7.16 (dd, J = 7.9, 2.2 Hz, 1 H, H-4′), 6.51 (dd, J = 17.1, 10.2 Hz, 1 H, C*H*=CH_2), 6.35 (dd, J = 17.1, 1.8 Hz, 1 H, CH=CH_2), 5.86 (dd, J = 10.1, 1.8 Hz, 1 H, CH=CH_2). Anal. ($C_{17}H_{13}ClN_4O\cdot0.25H_2O$) C, H, N.

***N*-[4-((3-Methylphenyl)amino)quinazolin-7-yl]acrylamide (3c).** A mixture of 3-methylaniline (223 mg, 2.1 mmol) and 4-chloro-7-nitroquinazoline[31] (420 mg, 2.0 mmol) in *i*-PrOH (10 mL) was refluxed for 16 h. On cooling, the solid was collected by Buchner filtration and air-dried to give the hydrochloride salt of 4-[(3-methylphenyl)amino]-7-nitroquinazoline[31] (**12**) as an acetone hemisolvate (401 mg, 58%): mp 230−233 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.06 (d, J = 9.0 Hz, 1 H, H-5), 8.95 (s, 1 H, H-2), 8.65 (d, J = 2.5 Hz, 1 H, H-8), 8.52 (dd, J = 9.0, 2.2 Hz, 1 H, H-6), 7.57 (m, 2 H, H-2′,6′), 7.38 (dd, J = 7.6, 8.6 Hz, 1 H, H-5′), 7.15 (d, J = 7.6 Hz, 1 H, H-4′), 2.37 (s, 3 H, Me). Anal. ($C_{15}H_{12}N_4O_2\cdot HCl\cdot0.5C_3H_6O$) C, H, N.

A solution of **12** (338 mg, 1.07 mmol) in MeOH (75 mL) was hydrogenated at 50 psi over Ra Ni (0.3 g) at 25 °C for 14.5 h. After isolation the crude product was dissolved in water and neutralized with dilute NaOH solution. The solid was collected and purified by column chromatography on silica gel, eluting with 5−10% MeOH in $CHCl_3$, to give 7-amino-4-[(3-methylphenyl)amino]quinazoline (**15**) (135 mg, 50%) as a pale-yellow solvated glass that was used directly (lit.[32] mp 196−197 °C): ^{1}H NMR [$(CD_3)_2SO$] δ 9.24 (brs, 1 H, NH), 8.35 (s, 1 H, H-2), 8.18 (d, J = 9.0 Hz, 1 H, H-5), 7.64 (m, 2 H, H-4′, 8), 7.22 (t, J = 7.8 Hz, 1 H, H-5′), 6.89−6.83 (m, 2 H, H-6, H6′), 6.69 (d, J = 2.2 Hz, 1 H, H-2′), 6.02 (brs, 2 H, NH_2), 2.32 (s, 3 H, Me).

Coupling of **15** with acrylic acid and EDCI.HCl in DMF and Et_3N (1.1 mmol) as above for 20 h and trituration of the crude product by sonication in a mixture of CH_2Cl_2/EtOAc/MeOH gave **3c** (75 mg, 49%): mp 269.5−270 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.63 (s, 1 H, CONH), 9.68 (s, 1 H, NH), 8.58 (s, 1 H, H-2), 8.54 (d, J = 9.3 Hz, 1 H, H-6), 8.25 (d, J = 2.2 Hz, 1 H, H-8), 7.83 (dd, J = 9.0, 1.9 Hz, 1 H, H-5), 7.71 (m, 2 H, H-2′,6′), 7.32 (t, J = 8.3 Hz, 1 H, H-5′), 6.99 (d, J = 7.1 Hz, 1 H, H-4′), 6.56, (dd, J = 16.8, 10.0 Hz, 1 H, C*H*=CH_2), 6.40 (dd, J = 17.1, 5.0 Hz, 1 H, CH=CH_2), 5.9 (dd, J = 10.3, 2.0 Hz, 1 H, CH=CH_2), 2.39 (s, 3 H, CH_3). Anal. ($C_{18}H_{16}N_4O\cdot0.5H_2O$) C, H, N.

***N*-[4-[(3-Bromophenyl)amino]pyrido[4,3-*d*]pyrimidin-7-yl]acrylamide (4a).** A stirred solution of 7-amino-4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidine[10] (**16**) (140 mg, 0.46 mmol), DMAP (14 mg), and Et_3N (excess, 2.0 mL) in THF/DMF (7:1, 17 mL) at 0 °C under N_2 was treated dropwise over 4 h with acryloyl chloride (4.8 mol equiv, 182 μL). The reaction was then stirred at 20 °C overnight before being worked up as above and chromatographed on silica gel. Elution with MeOH/CH_2Cl_2/EtOAc (5:45:50) gave **4a** (12 mg, 7%): mp (CH_2Cl_2/hexane) 215−220 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.15 (s, 1 H, CONH), 10.25 (s, 1 H, NH), 9.67 (s, 1 H, H-5), 8.71 (s, 1 H, H-2), 8.40 (s, 1 H, H-8), 8.21 (t, J = 1.9 Hz, 1 H, H-2′), 7.88 (dt, J = 7.6, 1.5 Hz, 1 H, H-6′), 7.38 (t, J = 7.7 Hz, 1 H, H-5′), 7.36 (dt, J = 7.7, 1.5 Hz, 1 H, H-4′), 6.68 (dd, J = 17.1, 10.2 Hz, 1 H, C*H*=CH_2), 6.39 (dd, J = 17.0, 1.8 Hz, 1 H, CH=CH_2), 5.86 (dd, J = 10.1, 1.8 Hz, 1 H, CH=CH_2). Anal. ($C_{16}H_{12}BrN_5O$) C, H.

***N*-[4-[(3-Bromophenyl)amino]quinazolin-6-yl]acrylamide (5a).** Acrylic acid (12.7 mmol, 0.87 mL) was added to a solution of 6-amino-4-[(3-bromophenyl)amino]quinazoline[21] (**23**) (2.0 g, 6.35 mmol) in dry DMF (20 mL) under N_2. The resulting solution was cooled to 0 °C, and EDCI·HCl (7.62 mmol, 1.46 g) was added. The reaction was stirred at 0 °C for 15 min and then allowed to warm to room temperature and stirred for a further 2 h, after which additional acrylic acid (0.30 mL) and EDCI.HCl (0.30 g) were added. After a further 2 h, the reaction was complete by TLC. Solvent was removed under reduced pressure, and the resulting residue was diluted with saturated $NaHCO_3$ and repeatedly extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Column chromatography on grade III alumina, eluting with EtOAc/MeOH (95:5), followed by recrystallization from EtOAc/hexane gave a spongy white solid, which upon several hours under high vacuum gave pure **5a** (1.06 g, 45%): mp 258−261 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.51 (s, 1 H, CONH), 9.93 (s, 1 H, NH), 8.83 (br s, 1 H, H-5), 8.59 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.94−7.78 (m, 3 H, H-6′,8,5′), 7.40−7.27 (m, 2 H, H-7&4′), 6.54 (dd, J = 17.0, 9.8 Hz, 1 H, C*H*=CH_2), 6.36 (dd, J = 16.9, 2.1 Hz, 1 H, CH=CH_2), 5.85 (dd, J = 9.7, 2.0 Hz, 1 H, CH=CH_2). Anal. ($C_{17}H_{13}BrN_4O$) C, H, N.

***N*-[4-[(3-Chlorophenyl)amino]quinazolin-6-yl]acrylamide (5b).** A mixture of 3-chloroaniline (5.10 g, 40 mmol) and 4-chloro-6-nitroquinazoline hydrochloride[31] (10 mmol) in *i*-PrOH (20 mL) was swirled at 25 °C for 10 min, producing a noticeable exotherm and a bright-yellow precipitate. The mixture was then stirred and refluxed for 3 h. On cooling, the

solid was collected by Buchner filtration, rinsed with *i*-PrOH (10 mL), and dried in a vacuum oven at 80 °C to give 4-[(3-chlorophenyl)amino]-6-nitroquinazoline (**17**) (2.35 g, 78%) as a yellow solid that was used directly (lit.[31] mp 272−274 °C): ^{1}H NMR [$(CD_3)_2SO$] δ 10.47 (brs, 1 H, NH) 9.64 (d, J = 2.2 Hz, 1 H, H-5), 8.77 (s, 1 H, H-2), 8.55 (dd, J = 2.1, 9.3 Hz, 1 H, H-7), 8.07 (t, J = 2.1 Hz, 1 H, H-2′), 7.94 (d, J = 9.3 Hz, 1 H, H-8), 7.84 (dd, J = 1.0, 8.1 Hz, 1 H, H-6′), 7.45 (t, J = 8.1 Hz, 1 H, H-5′), 7.23 (ddd, J = 1.0, 1.7, 8.1 Hz, 1 H, H-4′).

A solution of **17** (2.05 g, 6.8 mmol) in THF/MeOH (3:1, 100 mL) was hydrogenated at 52.5 psi over Ra Ni (4 g) at 25 °C for 22 h. Solvent was removed rigorously under reduced pressure, and the residual solid was dried under vacuum at 80 °C to give 6-amino-4-[(3-chlorophenyl)amino]quinazoline (**24**) (1.80 g, 98%) as a brown solid. An analytical sample was purified by preparative TLC (7% MeOH/$CHCl_3$): mp 186−189 °C (lit.[32] mp >150 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.47 (brs, 1 H, NH), 8.39 (s, 1 H, H-2), 8.12 (t, J = 2.0 Hz, 1 H, H-2′), 7.83 (ddd, J = 0.7, 1.9, 8.2 Hz, 1 H, H-6′), 7.55 (d, J = 8.7 Hz, 1 H, H-5), 7.38 (t, J = 8.1 Hz, 1 H, H-5′), 7.34 (d, J = 2.1 Hz, 1 H, H-5), 7.26 (dd, J = 2.3, 8.8 Hz, 1 H, H-7), 7.10 (ddd, J = 0.7, 1.9, 7.9 Hz, 1 H, H-4′), 5.64 (br s, 2 H, NH_2); MS CI (1% NH_3/MeOH) 273 (29, ^{37}ClMH$^+$), 271 (100, ^{35}ClMH$^+$). Anal. ($C_{14}H_{11}N_4$Cl) H, N; C: found, 61.7; required, 62.1.

A stirred solution of **24** (136 mg, 0.5 mmol), acrylic acid (74 mg, 1.0 mmol), and pyridine (200 mg, 2.5 mmol) in THF/DMF (4:1, 2.5 mL) was treated at 0 °C with EDCI.HCl (190 mg, 1 mmol) and then stirred at 25 °C for 3 h. The mixture was poured into water and extracted with EtOAc, and the organic layer was separated and washed with dilute HCl. The resulting precipitate was collected by filtration and washed with water to give **5b** as the HCl salt (93 mg, 48%): mp 223−227 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.46 (br s, 1 H, NH), 11.05 (s, 1 H, NH), 9.13 (d, J = 9.0, 2.0 Hz, 1 H, H-5), 8.90 (s, 1 H, H-2), 8.12 (dd, J = 9.0, 2.0 Hz, 1 H, H-7), 7.99 (d, J = 9.0 Hz, 1 H, H-8), 7.88 (t, J = 2.0 Hz, 1 H, H-2′), 7.68 (dd, J = 6.1, 1.0 Hz, 1 H, H-6′), 7.51 (t, J = 8.0 Hz, 1 H, H-5′), 7.37 (dd, J = 8.1, 1.2 Hz, 1 H, H-4′), 6.63 (dd, J = 17.1, 10.3 Hz, 1 H, C*H*=CH$_2$), 6.37 (dd, J = 17.1, 1.6 Hz, 1 H, CH=C*H*$_2$), 5.87 (dd, J = 10.1, 1.7 Hz, 1 H, CH=C*H*$_2$); MS CI (1% NH_3/MeOH) 327 (8, ^{37}ClMH$^+$), 325 (37, ^{35}ClMH$^+$). Anal. ($C_{18}H_{13}ClN_4O\cdot HCl\cdot 1.5H_2O$) C, H, N.

***N*-[4-[(3-Methylphenyl)amino]quinazolin-6-yl]acrylamide (5c).** A mixture of 3-methylaniline (16.0 g, 150 mmol) and 4-chloro-6-nitroquinazoline hydrochloride[31] (12.3 g, 50 mmol) in *i*-PrOH (100 mL) was swirled at 25 °C for 10 min. After the exotherm, the mixture was stirred under reflux for 2 h. On cooling, the solid was collected by Buchner filtration, rinsed with *i*-PrOH (2 × 25 mL), and dried in a vacuum oven at 60 °C to give 4-[(3-methylphenyl)amino]-6-nitroquinazoline[32] (**18**) (9.28 g, 66%) as a yellow solid: mp 250−252 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.40 (brs, 1 H, NH) 9.68 (d, J = 2.2 Hz, 1 H, H-5), 8.71 (s, 1 H, H-2), 8.56 (dd, J = 2.3, 9.1 Hz, 1 H, H-7), 7.93 (d, J = 9.1 Hz, 1 H, H-8), 7.68−7.62 (m, 2 H, H-2′, 6′), 7.31 (t, J = 8.0 Hz, 1 H, H-5′), 7.02 (d, J = 7.6 Hz, 1 H, H-4′), 2.36 (s, 3 H, Me); MS CI (1% NH_3/MeOH) 281 (100, MH$^+$). Anal. ($C_{15}H_{12}N_4O_2$) C, H, N.

Hydrogenation of **18** (9.27 g, 33 mmol) at 52.5 psi over Ra Ni (4 g) in THF/MeOH (3:2, 250 mL) at 25 °C for 3.5 h as above gave 6-amino-4-[(3-methylphenyl)amino]quinazoline (**25**) (8.14 mg, 97%) as a beige solid: mp 173−175.5 °C (lit.[32] mp 205−206 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 9.24 (brs, 1 H, NH), 8.35 (s, 1 H, H-2), 8.18 (d, J = 9.0 Hz, 1 H, H-5), 7.64 (m, 2 H, H-4′, 8), 7.22 (t, J = 7.8 Hz, 1 H, H-5′), 6.89−6.83 (m, 2 H, H-6, H-6′), 6.69 (d, J = 2.2 Hz, 1 H, H-2′), 6.02 (br s, 2 H, NH_2), 2.32 (s, 3 H, CH_3). Anal. ($C_{15}H_{14}N_4$) C, H, N.

Isobutyl chloroformate (20.35 g, 0.15 mol) was added dropwise over 20 min to a stirred solution of acrylic acid (10.82 g, 0.15 mol) and Et_3N (30.19 g, 0.30 mol) in THF (400 mL) under N_2 at 0 °C. The slurry was stirred at 0 °C for 30 min; then **25** (27.71 g, 107 mmol) in DMF (80 mL) was added dropwise over 45 min. After a further 4 h, an additional 0.05 mol of mixed anhydride was added in one portion, and the mixture was stirred for a further 15 min and then poured onto ice/water (1 L). The mixture was extracted with Et_2O, and the aqueous phase was extracted again with EtOAc. The combined organic extracts were washed with water and saturated brine, dried ($MgSO_4$), and adsorbed onto silica gel (150 g) by evaporation of the solvent. Flash chromatography of this on 700 g of silica gel, eluting with Me_2CO/CH_2Cl_2 (gradient from 25% to 40% Me_2CO), gave a crude product that was triturated in hot EtOAc by sonication (20 min at 60 °C). The resulting solid was collected by filtration and dried at 75 °C under vacuum for 16 h to give **5c** (11.38 g, 35%): mp 247−248 °C; ^{1}H NMR δ 10.49 (br s, 1 H, NH), 9.76 (br s, 1 H, NH), 8.75 (d, J = 2.5 Hz, 1 H, H-5), 8.52 (s, 1 H, H-2), 7.89 (dd, J = 9.2, 2.0 Hz, 1 H, H-7), 7.77 (d, J = 8.9 Hz, 1 H, H-8), 7.64−7.60 (m, 2 H, H2′,H-6′), 7.26 (td, J = 7.5, 1.4 Hz, 1 H, H-5′), 6.94 (d, J = 7.2 Hz, 1 H, H-4′), 6.53 (dd, J = 16.9, 10.1 Hz, 1 H, C*H*=CH$_2$), 6.34 (dd, J = 16.9, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.84 (dd, J = 10.1, 1.9 Hz, 1 H, CH=C*H*$_2$), 2.34 (s, 3 H, CH_3). Anal. ($C_{18}H_{16}N_4O\cdot 0.25H_2O$) C, H, N.

***N*-[4-[(3-Trifluoromethylphenyl)amino]quinazolin-6-yl]acrylamide (5d).** A solution of 6-amino-4-[(3-trifluoromethylphenyl)amino]quinazoline[8] (**26**) and acrylic acid in DMF/pyridine were treated with EDCI.HCl as above for 1 h. The solution was then cooled to 0 °C and treated with dilute HCl, and the resulting precipitate was collected by filtration, washed with water, and dried at 75 °C under vacuum for 12 h to give **5d** as the hydrochloride (87 mg, 45%): mp 195−199 °C; ^{1}H NMR δ 11.59 (br s, 1 H, NH), 10.99 (s, 1 H, NH), 9.17 (d, J = 2.0 Hz, 1 H, H-5), 8.92 (s, 1 H, H-2), 8.12 (s, 1 H, H-2′), 8.10 (dd, J = 9.2, 2.0 Hz, 1 H, H-7), 8.04 (d, J = 8.0 Hz, 1 H, H-6′), 7.98 (d, J = 9,0 Hz, 1 H, H-8), 7.74 (t, J = 7.9 Hz, 1 H, H-5′), 7.68 (d, J = 7.8 Hz, 1 H, H-4′), 6.60 (dd, J = 16.9, 10.1 Hz, 1 H, C*H*=CH$_2$), 6.38 (dd, J = 16.9, 1.6 Hz, 1 H, CH=C*H*$_2$), 5.89 (dd, J = 10.1, 1,6 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{18}H_{13}F_3N_4O\cdot HCl\cdot 0.5H_2O$) C, H, N.

***N*-[4-[(3-Bromo-4-fluorophenyl)amino]quinazolin-6-yl]acrylamide (5e).** Reaction of 4-chloro-6-nitroquinazoline[31] with 3-bromo-4-fluoroaniline according to the standard coupling procedure[21] gave 4-[(3-bromo-4-fluorophenyl)amino]-6-nitroquinazoline (**19**) (95%): mp (*i*-PrOH) 257−258.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.50 (s, 1 H, NH), 9.63 (d, J = 2.3 Hz, 1 H, H-5), 8.77 (d, J = 1.9 Hz, 1 H, H-2), 8.57 (dd, J = 9.2, 2.4 Hz, 1 H, H-7) 8.28 (td, J = 6.2, 2.6 Hz, 1 H, H-2′), 7.96 (d, J = 9.2 Hz, 1 H, H-8), 7.94−7.88 (m, 1H, H-6′), 7.46 (t, J = 8.8 Hz, H-5′). Anal. ($C_{14}H_8BrFN_4O_2$) C, H, N.

Iron dust reduction of **19** according to the general procedure[21] gave 6-amino-4-[(3-bromo-4-fluorophenyl)amino]quinazoline (**27**) (85%): mp (*i*-PrOH) 224−225.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.47 (s, 1 H, NH), 9.47 (s, 1 H, NH), 8.36 (s, 1 H, H-2), 8.31 (dd, J = 6.3, 2.4 Hz, 1 H, H-2′), 7.90−7.86 (m, 1 H, H-6′), 7.55 (d, J = 8.8 Hz, 1 H, H-8), 7.38 (t, J = 8.8 Hz, 1 H, H-5′), 7.31 (d, J = 1.7 Hz, 1 H, H-5), 7.31 (dd, J = 8.8, 1.9 Hz, 1 H, H-7), 5.63 (s, 2 H, NH_2). Anal. ($C_{14}H_{10}BrFN_4$) C, H, N.

A mixture of **27** (0.50 g, 1.5 mmol) and acrylic acid (0.325 g, 4.5 mmol) in DMA (15 mL) was cooled to 0 °C under nitrogen, and EDCI.HCl (0.865 g, 4.5 mmol) was added. The mixture was stirred at 0 °C for 15 min and allowed to warm to room temperature overnight. The crude product was extracted with EtOAc and eluted through a short column of silica gel with EtOAc to give **5e** (0.26 g, 45%) as a white solid: mp (MeOH) 276−278.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.51 (s, 1 H, NH), 9.95 (s, 1 H, NH), 8.82 (d, J = 1.8 Hz, 1 H, H-5), 8.57 (s, 1 H, H-2), 8.25 (td, J = 6.4, 2.6 Hz, 1 H, H-2′), 7.90−7.85 (m, 2 H, H-7, 6′), 7.81 (d, J = 8.9 Hz, 1 H, H-8), 7.41 (t, J = 8.8 Hz, 1 H, H-5′), 6.53 (dd, J = 17.0, 10.0 Hz, 1 H, C*H*=CH$_2$), 6.35 (dd, J = 17.0, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.85 (dd, J = 10.1, 1.9 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{17}H_{12}BrFN_4O$) C, H, N.

***N*-[4-[(3-Chloro-4-fluorophenyl)amino]quinazolin-6-yl]acrylamide (5f).** Reaction of 4-chloro-6-nitroquinazoline[31] with 3-chloro-4-fluoroaniline according to the standard coupling procedure[21] gave 4-[(3-chloro-4-fluorophenyl)amino]-6-nitroquinazoline (**20**) as its HCl salt (55%): mp (*i*-PrOH) 274.5−277 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.86 (d, J = 2.3 Hz, 1 H, H-5), 9.0 (s, 1 H, H-2), 8.76 (dd, J = 9.1, 1.6 Hz, 1 H, H-7) 8.15 (d, J = 9.0 Hz, 1 H, H-8), 8.09 (d, J = 7.6 Hz, H-2′), 7.82−7.75

(m, 1 H, H-6′), 7.58 (t, J = 9.0 Hz, H-5′). Anal. ($C_{14}H_8ClFN_4O_2$·HCl) C, H, N.

The free base of **20** (1.97 g, 62 mmol) in THF:MeOH (2:1, 600 mL) was hydrogenated at 50 psi over Ra Ni (10 g) for 3.5 h. The mixture was filtered, and the solvent was removed rigorously under reduced pressure to give 6-amino-4-[(3-chloro-4-fluorophenyl)amino]quinazoline[33] (**28**) (17.9 g, 100%) as a light-orange-brown solid. An analytical sample was obtained by preparative TLC on silica gel, eluting twice with 5% MeOH/$CHCl_3$: mp 263.5−265.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.47 (s, 1 H, NH), 9.48, (s, 1 H, NH, 8.36 (s, 1 H, H-2), 8.21 (dd, J = 6.7, 2.3 Hz, 1 H, H-2′), 7.83 (ddd, J = 2.8, 4.2, 9.0 Hz, 1 H, H-6′), 7.54 (d, J = 8.8 Hz, 1 H, H-8), 7.40 (t, J = 9.1 Hz, 1 H, H-5′), 7.31 (d, J = 2.2 Hz, 1 H, H-5), 7.25 (dd, J = 9.0, 2.1 Hz, 1 H, H-7), 5.64 (s, 2 H, NH_2). Anal. ($C_{14}H_{10}ClFN_4$) C, H, N.

EDCI·HCl (19.17 g, 0.1 mol) was added to a solution of **28** (14.435 g, 0.05 mol), acrylic acid (7.2 g, 0.1 mol), and pyridine (7.9 g, 0.1 mol) in THF (250 mL) and stirred under nitrogen at 0 °C. After 6 h, water (50 mL) was added to the reaction mixture with swirling, dissolving all of the gummy precipitate. This solution was poured slowly onto stirred ice−water, and the residual solid was collected slowly by Buchner filtration, rinsed with water (100 mL), and air-dried overnight. The greenish black solid was dissolved in the minimum of DMF (40 mL), added to a mixture of silica gel (150 g) Me_2CO (150 mL) for adsorption, and then subjected to flash chromatography on silica gel (500 g), eluting with a gradient of Me_2CO (0% to 60%) in CH_2Cl_2, to give **5f** (6.25 g, 35%) as a pale-yellow solid: 260−262 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.52 (s, 1 H, NH), 9.97 (s, 1 H, NH), 8.83 (d, J = 1.9 Hz, 1 H, H-5), 8.57 (s, 1 H, H-2), 8.15 (dd, J = 7.0, 2.6 Hz, 1 H, H-2′), 7.89 (dd, 1 H, H-7), 7.81 (d, J = 8.9 Hz, 1 H, H-8), 7.82−7.78 (m, 1 H, H-6′), 7.45 (t, J = 9.1 Hz, 1 H, H-5′), 6.53 (dd, J = 16.9, 10.1 Hz, 1 H, C*H*═CH_2), 6.35 (dd, J = 16.9, 1.9 Hz, 1 H, CH═C*H*$_2$), 5.85 (dd, J = 10.1, 1.9 Hz, 1 H, CH═C*H*$_2$). Anal. ($C_{17}H_{12}ClFN_4O$) C, H, N.

***N*-[4-[(4-Phenoxyphenyl)amino]quinazolin-6-yl]acrylamide (5g).** A suspension of crude 4-chloro-6-nitroquinazoline hydrochloride[31] (5 mmol), 4-phenoxyaniline (926 mg, 5 mmol), and *N*,*N*-dimethylaniline (1.215 g, 10 mmol) in 2-propanol (10 mL) was refluxed under nitrogen with stirring for 3 h. The mixture was allowed to cool to 25 °C, and the precipitate was collected by Buchner filtration, rinsed with 2-propanol (2 × 10 mL), and dried at 60 °C in a vacuum oven to give 6-nitro-4-[(4-phenoxyphenyl)amino]quinazoline hydrochloride[25] (**21**) (1.70 g, 86%) as an orange solid: mp 293−294 °C (lit.[25] mp >200 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 12.00 (br s, 1 H, NH), 9.88 (d, J = 2.4 Hz, 1 H, H-5), 8.97 (s, 1 H, H-2), 8.76 (dd, J = 2.3, 9.1 Hz, 1 H, H-7), 8.16 (d, J = 9.3 Hz, 1 H, H-8), 7.76 (d, J = 9.O Hz, 2 H, H-2′,6′), 7.44 (dd, J = 7.6, 8.8 Hz, 2 H, H-3′,5′), 7.18 (t, J = 7.3 Hz, 1 H, H-4′), 7.14 (d, J = 9.0 Hz, 2 H, H-2″,6″), 7.08 (d, J = 7.6 Hz, 2 H, H-3″,5″); MS (APCI) 359 (100 MH^+). Anal. ($C_{20}H_{14}N_4O_3$·HCl) C, H, N.

A solution of **21** (1.65 g, 4.2 mmol) in MeOH/THF (1:1, 100 mL) was hydrogenated over Raney nickel (1 g) at 50 psi and 27 °C for 18 h. The reaction mixture was filtered through Celite, and the volatiles were removed under reduced pressure. The residue was partially redissolved in MeOH (25 mL), and dilute aqueous Na_2CO_3 solution (0.1 M, 50 mL) was added with vigorous stirring. After 2 h, the precipitate was collected by Buchner filtration, rinsed with water (50 mL), air-dried, and purified by flash chromatography on silica gel, eluting with 2.5% and then 4% MeOH in CH_2Cl_2, to give 6-amino-4-[(4-phenoxyphenyl)amino]quinazoline[25] (**29**) (1.113 g, 80%) as a pale-yellow glassy foam: mp 89−90 °C, remelt 195−200 °C (lit.[25] mp 187−191 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 9.38 (brs, 1 H, NH), 8.30 (s, 1 H, H-2), 7.87 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.40 (d, J = 8.8 Hz, 1 H, H-8), 7.38 (dd, J = 7.3, 8.6 Hz, 2 H, H-3″,5″), 7.34 (d, J = 2.4 Hz, 1 H, H-5), 7.23 (dd, J = 2.4, 8.9 Hz, 1 H, H-7), 7.11 (t, J = 7.4 Hz, 1 H, H-4″), 7.05 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.00 (d, J = 7.8 Hz, 2 H, H-2″,6″), 5.58 (br s, 2 H, NH_2); MS (APCI) 329 (100 MH^+). Anal. ($C_{20}H_{16}N_4O$·0.25H_2O) C, H, N.

A solution of **29** (328 mg, 1.0 mmol), acrylic acid (148 mg, 2.04 mmol), and pyridine (165 mg, 2.1 mmol) in THF (10 mL) was treated with EDCI.HCl (385 mg, 2.0 mmol) (in one portion), and the mixture was stirred under nitrogen at 0 °C for 4 h and at 25 °C for 2 h. The reaction was then cooled to 0 °C, and water (2 mL) was added dropwise. This solution was poured onto rapidly stirred ice−water (40 mL), the pH was raised to 7 with saturated Na_2CO_3 solution, and the very fine precipitate was collected by Buchner filtration. The solid was rinsed with water (2 × 10 mL), dried in a vacuum oven at 65 °C for 4 h, and then dissolved (heating and sonication) in EtOAc (10 mL). The solution was filtered, and the filtrate was eluted through a small silica gel plug in EtOAc to give **5g** (162 mg, 41%) as a pale-yellow glass: mp 185−191 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.50 (br s, 1 H, NH), 9.86 (br s, 1 H, NH), 8.80 (d, J = 2.0 Hz, 1 H, H-5), 8.50 (s, 1 H, H-2), 7.89 (dd, J = 2.2, 9.0 Hz, 1 H, H-7), 7.81 (d, J = 8.9 Hz, 2 H, H-2′,6′), 7.77 (d, J = 9.0 Hz, 1 H, H-8), 7.39 (dd, J = 7.6, 8.6 Hz, 2 H, H-3″,5″), 7.12 (t, J = 7.4 Hz, 1 H, H-4″), 7.06 (d, J = 8.8 Hz, 2 H, H-3′,5′), 7.02 (d, J = 7.8 Hz, 2 H, H-2″,6″), 6.53 (dd, J = 10.1, 17.0 Hz, 1 H, C*H*═CH_2), 6.34 (dd, J = 1.8, 17.0 Hz, 1 H, CH═C*H*$_2$), 5.84 (dd, J = 1.8, 10.1 Hz, 1 H, CH═C*H*$_2$); MS (APCI) 383.1 (100 MH^+). Anal. ($C_{23}H_{18}N_4O_2$·0.25HCl·0.06$C_4H_6O_2$) C, H, N.

***N*-[4-[(4-Benzyloxyphenyl)amino]quinazolin-6-yl]acrylamide (5h).** A suspension of crude 4-chloro-6-nitroquinazoline hydrochloride[31] (5 mmol), 4-benzyloxyaniline (999 mg, 5 mmol), and *N*,*N*-dimethylaniline (1.209 g, 10 mmol) in 2-propanol (10 mL) was refluxed under nitrogen with stirriing for 3 h. The mixture was cooled, and the precipitate was collected by Buchner filtration, rinsed with 2-propanol (2 × 10 mL), and dried to give 4-[(4-benzyloxyphenyl)amino]-6-nitroquinazoline hydrochloride[25] (**22**) (1.675 g, 82%) as a yellow solid: mp 246−248 °C (lit.[25] mp 222−223 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 11.98 (br s, 1 H, NH), 9.85 (d, J = 2.2 Hz, 1 H, H-5), 8.94 (s, 1 H, H-2), 8.76 (dd, J = 2.3, 9.1 Hz, 1 H, H-7), 8.15 (d, J = 9.3 Hz, 1 H, H-8), 7.65 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.48 (d, J = 7.0 Hz, 2 H, H-2″,6″),7.41 (t, J = 7.4 Hz, 2 H, H-3″,5″), 7.31 (t, J = 7.4 Hz, 1 H, H-4″), 7.15 (d, J = 9.1 Hz, 2 H, H-3), 5.16 (s, 2 H, benzylic); MS (APCl) 373 (100 MH^+). Anal. ($C_{21}H_{l6}N_4O_3$·HCl) C, H, N.

A solution of **22** (1.46 g, 3.57 mmol) in MeOH/THF (1:1, 100 mL) was hydrogenated over Raney nickel (1 g) at 51.5 psi and 23 °C for 18 h. The reaction mixture was filtered, and the volatiles were removed under reduced pressure. The residue was partially redissolved in MeOH (25 mL), and dilute aqueous Na_2CO_3 solution (0.1 M, 50 mL) was added with vigorous stirring. After 2 h the precipitate was collected by Buchner filtration, rinsed with water (50 mL), dried in a vacuum oven at 60 °C, and then purified by flash chromatography on silica gel, eluting with $CHCl_3$ and then 4% MeOH in $CHCl_3$ to give 6-amino-4-[(4-benzyloxyphenyl)amino]quinazoline[25] (**30**) (1.018 g, 80%) as a cream powder: mp l73−175 °C, remelt 240−245 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.25 (br s, 1 H, NH), 8.25 (s, 1 H, H-2), 7.70 (d, J = 9.0 Hz, 2 H, H-2′,6′), 7.52−7.47 (m, 3 H, H-2″,6″,5), 7.41 (t, J = 7.5 Hz, 2 H, H-3″,5″), 7.38−7.35 (m, 2 H, H-7,8), 7.02 (d, J = 9.0 Hz, 2 H, H-3′,6′), 5.54 (br s, 2 H, NH_2), 5.11 (s, 2 H, benzylic); MS (APCI) 343 (100, MH^+). Anal. ($C_{20}H_{16}N_4O$·0.67H_2O) C, H, N.

EDCI·HCl (385 mg, 2.0 mmol) was added in one portion to a solution of **30** (342 mg, 1. 0 mmol), acrylic acid (144 mg, 2.0 mmol), and pyridine (163 mg, 2.06 mmol) in THF (10 mL) and stirred under nitrogen at 0 °C. After 4 h at 0 °C and a further 2 h at 25 °C, the mixture was recooled to 0 °C, and water (2 mL) was added dropwise. This solution was poured onto rapidly stirred ice−water (40 mL); the pH was raised to 7 with saturated Na_2CO_3 solution. The precipitate was collected by Buchner filtration, rinsed with water (2 × 10 mL), and dried in a vacuum oven at 65 °C for 4 h and then dissolved in $CHCl_3$/Me_2CO (1:1, 40 mL). This was adsorbed onto silica gel (5 g) and used as the origin of a silica gel flash chromatography column, eluting with 25% Me_2CO/$CHC1_3$, to give **5h** (152 mg, 38%): mp 227−229 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.47 (br s, 1 H, NH), 9.74 (br s, 1 H, NH), 8.76 (d, J = l.9 Hz, 1 H, H-5), 8.45 (s, 1 H, H-2), 7.86 (dd, J = 2.0, 9.0 Hz, 1 H, H-7), 7.74 (d, J =

8.9 Hz, 1 H, H-8), 7.66 (d, J = 9.2 Hz, 2 H, H-2′,6′), 7.49 (d, J = 7.0 Hz, 2 H, H-2″,5″), 7.41 (t, J = 7.4 Hz, 2 H, H-3″,5″), 7.34 (t, J = 7.2 Hz, l H, H-4″), 7.04 (d, J = 9.2 Hz, 2 H, H-3′,5′), 6.53 (dd, J = 10.1, 16.9 Hz, 1 H, C*H*=CH_2), 6.34 (dd, J = 1.9, 16.9 Hz, 1 H, CH=C*H*$_2$), 5.83 (dd, J = 1.9, 10.1 Hz, 1 H, CH=C*H*$_2$), 5.13 (s, 2 H, benzylic); MS (APCI) 397.2 (100 MH^+). Anal. ($C_{24}H_{20}N_4O_2$·0.1HCl) C, H, N.

***N*-[4-[(3-Bromophenyl)amino]pyrido[3,4-*d*]pyrimidin-6-yl]acrylamide (6a).** A solution of 6-amino-4-[(3-bromophenyl)amino]pyrido[3,4-*d*]pyrimidine[11] (**31**) (520 mg, 1.64 mmol), redistilled acrylic acid (0.4 mL, 3.5 equiv), and pyridine (1.64 mL, 12.4 equiv) in THF/DMA (2:1, 6.6 mL) purged with nitrogen was cooled to 0−5 °C and treated with EDCI.HCl (1.61 g, 5 equiv) in one portion. The resulting suspension was kept at 0−5 °C for 30 min and then brought to 25 °C. After 2.5 h, the solution was ice-cooled and treated slowly with an excess of water (ca. 10−12 mL). After the thick suspension stirred for about 30 min, the solids were collected, washed well with water, and air-dried. The solids were suspended in ca. 15 mL of *i*-PrOH, and the stirred suspension was heated at 70−80 °C for 30 min. After cooling, the solids were collected, washed with *i*-PrOH, and dried to give **6a** (360 mg, 59%): mp 243−245 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.07 (s, 1 H, CONH), 10.33 (s, 1 H, NH), 9.05 (s, 1 H, aromatic), 9.03 (s, 1 H, aromatic), 8.66 (s, 1 H, aromatic), 8.18 (br s, 1 H, H-2′), 7.89 (br d, J = 7.6 Hz, 1 H, H-6′). 7.40−7.33 (m, 2 H, H-4′,5′), 6.70 (dd, J = 17.0, 10.2 Hz, 1 H, C*H*=CH_2), 6.41 (dd, J = 16.9, 1.2 Hz, 1 H, CH=C*H*$_2$), 5.87 (dd, J = 10.1, 1,2 Hz, 1 H, CH=C*H*$_2$); ^{13}C NMR δ 163.35, 156.82, 154.13, 150.87, 147.92, 141.64, 140.40, 131.25, 130.26, 127.86, 126.49, 124.76, 121.30, 121.02, 120.97, 103.43; CIMS *m*/*z* (relative %) 369 (29), 370 (100), 371 (46), 372 (95), 373 (17). Anal. ($C_{16}H_{12}N_5OBr$) Calcd: C, 51.9; H, 3.3; N, 18.9. Found: C, 51.8; H, 3.1; N, 18.6.

The filtrate was evaporated to dryness and dissolved in EtOAc/*i*-PrOH (9:1), and the solution was filtered through a pad of flash silica gel, eluting with EtOAc. Further processing of the filtrate as above provided a second crop of **6a** (57 mg, 9%): mp 240−243 °C.

***N*-[4-[(3-Methylphenyl)amino]pyrido[3,4-*d*]pyrimidin-6-yl]acrylamide (6c).** To a stirred solution of 6-amino-4-[(3-methylphenyl)amino]pyrido[3,4-*d*]pyrimidine[20] (**32**) (140 mg, 0.56 mmol), DMAP (14 mg), and Et_3N (excess, 0.5 mL) in THF/DMF (7:1, 20 mL) at 0 °C under N_2 was added acryloyl chloride (2.7 mol equiv, 123 μL) dropwise over 3 h. The reaction was then stirred at 20 °C for 1 h, diluted with water, and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure before being chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (2:48:50) to give **6c** (41 mg, 24%): mp (EtOAc/hexane) 221−223 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.03 (s, 1 H, CONH), 10.18 (s, 1 H, NH), 9.02 (s, 1 H, aromatic), 9.01 (s, 1 H, aromatic), 8.59 (s, 1 H, aromatic), 7.63 (m, 2 H, H-2′,6′), 7.29 (m, 1 H, H-5′), 6.89 (br d, J = 7.5 Hz, 1 H, H-4′), 6.69 (dd, J = 17.0, 10.2 Hz, 1 H, C*H*=CH_2), 6.37 (dd, J = 17.0, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.85 (dd, J = 10.2, 1.9 Hz, 1 H, CH=C*H*$_2$), 2.35 (s, 3 H, CH_3-Ar). Anal. ($C_{17}H_{15}N_5O$) C, H, N.

***N*-[4-[(3-Bromo-4-fluorophenyl)amino]pyrido[3,4-*d*]pyrimidin-6-yl]acrylamide (6e).** Reaction of 4-chloro-6-fluoropyrido[3,4-*d*]pyrimidine[20] with 3-bromo-4-fluoroaniline, by the standard procedure,[20] gave 4-[(3-bromo-4-fluorophenyl)-amino]-6-fluoropyrido[3,4-*d*]pyrimidine (**40**) (59%): mp (*i*-PrOH) 269−270 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.13 s, 1 H, NH), 8.97 (s, 1 H, H-8), 8.74 (s, 1 H, H-2), 8.33 (td, J = 6.0, 2.6 Hz, H-2′), 8.23 (br s, 1 H, H-5), 7.93−7.87 (m, 1 H, H-6′), 7.46 (t, J = 8.8 Hz, 1 H, H-5′). Anal. ($C_{13}H_7BrF_2N_4$) C, H, N.

A mixture of **40** (2.03 g, 6 mmol) and 4-methoxybenzylamine (16.5 g, 0.12 mol) in DMSO (50 mL) was heated at 100 °C for 16 h. The mixture was diluted with water and extracted with EtOAc to give a crude solid which was chromatographed on silica gel, eluting with EtOAc/CH_2Cl_2 (1:1), to give 4-[(3-bromo-4-fluorophenyl)amino]-6-[(4-methoxyphenyl)methylamino]pyrido[3,4-*d*]pyrimidine (**42**) (2.4 g, 88%): ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H, NH), 8.75 (s, 1H, H-8), 8.38 (s, 1 H, H-2), 8.26 (dd, J = 6.4, 2.6 Hz, 1 H, H-2′), 7.89−7.85 (m, 1 H, H-6′), 7.42 (t, J = 8.8 Hz, 1 H, H-5′), 7.33 (d, J = 8.5 Hz, 2 H, H-2″,6″), 7.31 (br s, 1 H, NH), 7.19 (s, 1 H, H-5), 6.89 (br d, J = 8.7 Hz, 2 H, H-3″,5″), 4.49 (d, J = 6.2 Hz, 2 H, CH_2), 3.71 (s, 3 H, OMe).

Without further purification, **42** was dissolved in TFA (50 mL), and the solution was refluxed for 30 min. The TFA was removed under reduced pressure; the residue was made basic with aqueous NH_3 and extracted into EtOAc. After drying and removal of solvent, the residue was chromatographed on silica gel, eluting with EtOAc/MeOH (98:2), to give 6-amino-4-[(3-bromo-4-fluorophenyl)amino]pyrido[3,4-*d*]pyrimidine (**33**) (1.61 g, 91%): mp (EtOAc) 268−270 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (s, 1 H, NH), 8.71 (s, 1 H, H-8), 8.38 (d, J = 1.4 Hz, 1 H, H-2), 8.32 (dd, J = 6.5, 2.6 Hz, 1 H, H-2′), 7.92−7.87 (m, 1 H, H-6′), 7.41 (t, J = 8.8 Hz, 1 H, H-5′), 7.13 (s, 1 H, H-5), 6.31 (br s, 2 H, NH_2). Anal. ($C_{13}H_9BrFN_5$) C, H, N.

A stirred solution of **33** (1.705 g, 5.1 mmol) in dry pyridine (26 mL) at 0−5 °C was treated with redistilled acrylic acid (1.4 mL, 20.4 mmol), followed by EDCI.HCl (4.9 g, 25.5 mmol). The mixture was stirred for 3 h; then the viscous suspension was poured into 200 mL of ice-cold 2% aqueous $NaHCO_3$ (using some DMF to assist in the transfer). The precipitate was collected, washed well with water, then suspended in water, and sonicated for 15 min. The solids were collected, washed with H_2O, and air-dried. Upon standing overnight, the combined aqueous filtrates precipitated additional solids, which were collected as above. The two crops were combined and suspended in 70 mL of hot EtOAc. After heating at reflux for 2 h, the suspension was cooled and the solids were collected to give **6e** (1.65 g, 80%) as a pale-yellow solid: mp (MeOH) 245−248 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.07 (s, 1 H, NH), 10.35 (s, 1 H, NH), 9.04 and 9.01 (2s, 2 H, H-5 and H-8), 8.64 (s, 1 H, H-2), 8.25 (dd, J = 6.4, 2.6 Hz, 1 H, H-2′), 7.91−7.87 (m, 1 H, H-6′), 7.43 (t, J = 8.8 Hz, 1 H, H-5′), 6.70 (dd, J = 17.0, 10.1 Hz, 1 H, C*H*=CH_2), 6.39 (dd, J = 17.0, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.86 (dd, J = 10.1, 1.9 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{16}H_{11}BrFN_5O$) C, H, N.

***N*-[4-[(3-Chloro-4-fluorophenyl)amino]pyrido[3,4-*d*]pyrimidin-6-yl]acrylamide (6f).** A mechanically stirred solution of 4-chloro-6-fluoropyrido[3,4-*d*]pyrimidine[20] (7.62 g, 41.5 mmol) and 3-chloro-4-fluoroaniline (7.3 g, 49.9 mmol) in *i*-PrOH (120 mL) was heated at reflux for 3 h. The resulting suspension was concentrated to ca. 50 mL, and the thick yellow precepitate was collected and washed with a little *i*-PrOH. The filter cake was further washed with 2% aqueous NaOH to pH 12 and water to neutral pH and then dried at 70 °C/2 mmHg over P_2O_5 for 4 days to give 4-[(3-chloro-4-fluorophenyl)amino]-6-fluoropyrido[3,4-*d*]pyrimidine[34] (**41**) (9.65 g, 79%): mp 267−269 °C (lit.[34] mp 264−266 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 10.10 (s, 1 H, NH), 8.91 (s, 1 H, H-8), 8.68 (s, 1 H, H-2), 8.18 (br s, 2 H, H-5, H-2′), 7.79−7.75 (m, 1 H, H-6′), 7.44 (t, J = 9.1 Hz, 1 H, H-5′). Anal. ($C_{13}H_7N_4F_2Cl$) C, H, N, F, Cl.

A solution of **41** (9.6 g, 32.8 mmol) and 4-methoxybenzylamine (17.1 mL, 131 mmol) in DMSO (66 mL) was heated at 120 °C for 16 h. Most of the DMSO and excess amine were removed under reduced pressure at 80 °C, and the residual yellow solid was dissolved in minimum hot EtOAc. Upon storage in the cold overnight, the solids that had precipitated were collected, washed with cold EtOAc, and dried to give 4-[(3-chloro-4-fluorophenyl)amino]-6-[(4-methoxyphenyl)methylamino]pyrido[3,4-*d*]pyrimidine (**43**) (6.77 g, 50%): mp (EtOAc) 196−197 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.74 (s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.38 (s, 1 H, H-2), 8.18 (dd, J = 6.9, 2.5 Hz, 1 H, H-2′), 7.84−7.79 (m, 1 H, H-6′), 7.46 (t, J = 8.9 Hz, 1 H, H-5′), 7.36−7.32 (d, J = 8.7 Hz + overlapping br s, 3 H, H-2″,6″ and NH), 7.17 (s, 1 H, H-5), 6.88 (br d, J = 8.7 Hz, 2 H, H-3″,5″), 4.49 (d, J = 6.3 Hz, 2 H, CH_2), 3.71 (s, 3 H, OCH_3). Anal. ($C_{21}H_{17}N_5OClF$) C, H, N, Cl, F.

Processing of the EtOAc filtrate by concentration, dissolution of the residue in a minimum amount of solvent, and storage in the cold yielded 2.41 g (~20%) of additional product in two crops. TLC showed this to be predominately **35** along with a smaller amount (10−20%) of a lower R_f spot that

corresponded to 6-amino-4-[(3-chloro-4-fluorophenyl)amino]-pyrido[3,4-*d*]pyrimidine (**34**). This material was suitable for direct use in the next reaction.

A solution of **43** (9.0 g, 22 mmol) in trifluoroacetic acid (58 mL) was heated at 50 °C for 1 h, and the mixture was evaporated to dryness. The residue was coevaporated three times with MeOH and then dissolved in a minimum volume of DMF. The cooled solution was diluted with 175 mL of ethyl acetate and then washed with 125 mL of 5 M aqueous NH_4-OH solution. Upon standing at 25 °C, the organic phase deposited a precipitate that was collected, washed with EtOAc, and dried to give 6-amino-4-[(3-chloro-4-fluorophenyl)amino]-pyrido[3,4-*d*]pyrimidine (**34**) (2.7 g, 42%): mp (EtOAc) 263−266 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.81 (s, 1 H, NH), 8.71 (s, 1 H, H-8), 8.38 (s, 1 H, H-2), 8.23 (dd, J = 6.8, 2.2 Hz, 1 H, H-2′), 7.86−7.82 (m, 1 H, H-6′), 7.45 (t, J = 9.2 Hz, 1 H, H-5′), 7.13 (s, 1 H, H-5), 6.33 (br s, 2 H, NH_2); ^{19}F NMR δ −123.0 (F-4′). Anal. ($C_{13}H_9N_5FCl\cdot0.25H_2O$) C, H, N, Cl, F.

Processing of the filtrate by concentration followed by dissolution in a minimum volume of EtOAc and crystallization afforded 2.6 g (40%) of slightly less pure **26** in two crops.

A stirred solution of **34** (4.64 g, 16 mmol) in dry pyridine (82 mL) at 0−5 °C was treated with redistilled acrylic acid (4.5 mL, 64 mmol), followed by EDCI.HCl (15.3 g, 80 mmol). The mixture was stirred for 45 min; then the viscous suspension was poured into 500 mL of ice-cold 2% aqueous $NaHCO_3$. The precipitate was collected, washed well with water, then suspended in water, and sonicated for 15 min. The solids were collected, washed with water, and air-dried. Upon standing overnight, the combined aqueous filtrates precipitated additional solids which were collected as above. The two crops were combined and suspended in hot EtOAc (200 mL). After heating at reflux for 1.5 h, the suspension was cooled and the solids were collected to give **6f** (4.31 g, 75%) as a pale-yellow powder: mp >245 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.03 (s, 1 H, NH), 10.31 (s, 1 H, NH), 8.98 & 8.95 (2xs, 2 H, H-5, H-8), 8.59 (s, 1 H, H-2), 8.09 (dd, J = 6.0, 2.1 Hz, 1 H, H-2′), 7.80−7.74 (m, 1 H, H-6′), 7.41 (t, J = 9.0 Hz, 1 H, H-5′), 6.64 (dd, J = 16.9, 10.2 Hz, 1 H, C*H*=CH_2), 6.33 (dd, J = 16.9, 1.0 Hz, 1 H, CH=C*H*$_2$), 5.80 (dd, J = 10.2, 1.0 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{16}H_{11}N_5OClF\cdot H_2O$) C, H, N.

***N*-[4-[(3-Bromophenyl)amino]pyrido[3,2-*d*]pyrimidin-6-yl]acrylamide (7a).** To a stirred solution of 6-amino-4-[(3-bromophenyl)amino]pyrido[3,2-*d*]pyrimidine[11] (**35**) (46 mg, 0.15 mmol) and acrylic acid (6 mol equiv, 0.91 mmol, 62 μL) in DMA (5.0 mL) under N_2 was added EDCI.HCl (4.0 mol equiv, 0.61 mmol, 116 mg). The reaction mixture was stirred for 48 h with additional amounts of acrylic acid and EDCI.HCl (62 μL/116 mg) being added every 12 h. It was then poured into water and extracted with EtOAc, and the product was chromatographed on silica gel, eluting with EtOAc:CH_2Cl_2 (1:1) to MeOH/CH_2Cl_2/EtOAc (2:48:50), to give **7a** (14 mg, 26%): mp (CH_2Cl_2/hexane) 226−228 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.13 (s, 1 H, CONH), 9.57 (s, 1 H, NH), 8.72 (s, 1 H, aromatic), 8.69 (d, J = 9.1 Hz, 1 H, H-5 or H-6), 8.43 (t, J = 1.9 Hz, 1 H, H-2′), 8.30 (d, J = 9.1 Hz, 1 H, H-5 or H-6), 7.87 (br d, J = 6.9 Hz, 1 H, H-6′), 7.39 (t, J = 8.1 Hz, 1 H, H-5′), 7.33 (dt, J = 8.2, 1.3, 1.3 Hz, 1 H, H-4′), 6.68 (dd, J = 17.0, 10.2, Hz, 1 H, C*H*=CH_2), 6.43 (dd, J = 17.0, 1.8 Hz, 1 H, CH=C*H*$_2$), 5.91 (dd, J = 10.2, 1.8 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{16}H_{12}BrN_5O$) C, H, N.

***N*-[4-[(3-Bromo-4-fluorophenyl)amino]pyrido[3,2-*d*]pyrimidin-6-yl]acrylamide (7e).** 4-Chloro-6-fluoropyrido[3,2-*d*]pyrimidine[11] (1.10 g, 82%) was dissolved in EtOH (140 mL) containing 3-bromo-4-fluoroaniline (2.54 g, 0.013 mol) and concentrated HCl (2 drops), and the solution was refluxed for 15 min. After concentration under reduced pressure to a volume of ca. 40 mL, saturated $NaHCO_3$ solution was added to precipitate the product. This was chromatographed on silica gel, eluting with EtOAc/petroleum ether, to give crude [4-[(3-bromo-4-fluorophenyl)amino]-6-fluoropyrido[3,2-*d*]pyrimidine (**44**) as a yellow powder (1.35 g, 58%), which was used directly. A solution of **44** (0.47 g, 1.48 mmol) in EtOH (50 mL) was saturated with ammonia gas and heated in a pressure vessel at 110 °C for 18 h. The solution was concentrated to dryness directly onto silica and chromatographed. Elution with EtOAc/petroleum ether (1:1) gave foreruns, while EtOAc gave 6-amino-4-[(3-bromo-4-fluorophenyl)amino]pyrido[3,2-*d*]pyrimidine (**36**) (0.30 g, 61%): mp (EtOH) 316−317 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.40 (s, 1H, NH), 8.51 (dd, J = 6.45, 2.3 Hz, 1 H, H-2′), 8.44 (s, 1 H, H-2), 7.94−7.90 (m, 1 H, H-6′), 7.84 (d, J = 9.0 Hz, 1 H, H-8), 7.39 (dd, J = 8.8, 8.8 Hz, H-5′), 7.13 (d, J = 9.0 Hz, 1 H, H-7), 6.75 (br s, 2 H, NH_2). Anal. ($C_{13}H_9BrFN_5$) C, H, N.

Acrylic acid (0.32 mL, 6.31 mmol) and EDCI (1.72 g, 8.97 mmol) were added to a stirred solution of **36** (0.60 g, 1.79 mmol) and pyridine (1.74 mL, 0.021 mol) in DMF (30 mL). Stirring was continued at room temperature for 7 days, with further quantities of acrylic acid (0.12 mL), pyridine (0.70 mL), and EDCI (0.66 g) being added every 48 h. The solution was concentrated to a volume of 10 mL, and water was added to precipitate the product. The crude material was adsorbed onto silica from a methanol solution and chromatographed. EtOAc eluted **7e**, which was slurried in ether to give pure material (0.25 g, 39%): mp 241−243 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.10 (s, 1H, CONH), 9.63 (s, 1 H, NH), 8.68 (d, J = 9.2 Hz, 1 H, H-8), 8.49 (dd, J = 6.3, 2.6 Hz, 1 H, H-2′), 8.28 (d, J = 9.2 Hz, 1 H, H-7), 7.94−7.90 (m, 1 H, H-6′), 7.45 (dd, J = 8.8, 8.8 Hz, 1 H, H-5′), 6.68 (dd, J = 17.1, 10.1 Hz, 1 H, C*H*=CH_2), 6.42 (dd, J = 17.1, 1.7 Hz, 1 H, CH=C*H*$_2$), 5.91 (dd, J = 10.1, 1.7 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{16}H_{11}BrFN_5O$) C, H, N.

***N*-[4-[(3-Chloro-4-fluorophenyl)amino]pyrido[3,2-*d*]pyrimidin-6-yl]acrylamide (7f).** Reaction of 4-chloro-6-fluoropyrido[3,2-*d*]pyrimidine[11] with 3-chloro-4-fluoroaniline as above gave crude [4-[(3-chloro-4-fluorophenyl)amino]-6-fluoropyrido-[3,2-*d*]pyrimidine (**45**) (86%) which was used directly. A solution of **45** (0.45 g, 1.70 mmol) in propan-2-ol (30 mL) was saturated with gaseous ammonia and heated in a pressure vessel at 100 °C for 18 h. On cooling crude product precipitated out. This was redissolved in MeOH, adsorbed onto silica gel, and chromatographed. EtOAc eluted foreruns, while EtOAc/MeOH (9:1) gave 6-amino-4-[(3-chloro-4-fluorophenyl)amino]-pyrido[3,2-*d*]pyrimidine (**37**) (0.29 g, 59%): mp (EtOAc) 300−303 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.41 (s, 1 H, NH), 8.44 (s, 1 H, H-2), 8.41 (d, J = 6.1 Hz, 1 H, H-2′), 7.88 (m, 1 H, H-6′), 7.83 (d, J = 8.9 Hz, 1 H, H-8), 7.42 (dd, J = 9.0, 9.0 Hz, 1 H, H-5′), 7.13 (d, J = 8.9 Hz, 1 H, H-7), 6.75 (br s, 2 H, NH_2). Anal. ($C_{13}H_9ClFN_5\cdot0.5H_2O$) C, H, N.

Reaction of **37** with acrylic acid and EDCI over 7 days as described above gave **7f** (57%): mp (EtOAc) 247 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.09 (s, 1 H, NHCO), 9.61 (s, 1 H, NH), 8.68 (d, J = 9.2 Hz, 1 H, H-8), 8.67 (s, 1 H, H-2), 8.40−8.37 (m, 1 H, H-2′), 8.27 (d, J = 9.2 Hz, 1H, H-7), 7.88−7.84 (m, 1 H, H-6′), 7.48 (dd, J = 9 0.0, 9.0 Hz, 1H, H-5′), 6.69 (dd, J = 17.0, 10.1 Hz, 1 H, C*H*=CH_2), 6.42 (dd, J = 17.0, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.90 (dd, J = 10.1, 1.9 Hz, 1 H, CH=C*H*$_2$). Anal. ($C_{16}H_{11}FN_5O$) C, H, N.

***N*-[4-[1-(6-Bromoindolinyl)]quinazolin-6-yl]acrylamide (8).** A suspension of 6-bromoindoline[35] (490 mg, 2.47 mmol), 4-chloro-6-nitroquinazoline[31] (660 mg, 2.47 mmol), and *N*,*N*-dimethylaniline (0.63 mL, 4.9 mmol) in 2-propanol (15 mL) was heated under reflux for 1 h. The suspension was concentrated to give a solid, which was shaken with a mixture of EtOAc and saturated aqueous $NaHCO_3$, collected by filtration, and recrystallized from a boiling mixture of EtOH (100 mL) and DMF (25 mL) to give 4-[1-(6-bromoindolinyl)]-6-nitroquinazoline (**38**) (290 mg, 32%): mp 249−251 °C; ^{1}H NMR (CF_3CO_2D) δ 9.45 (s, 1 H, H-5), 8.90 (dd, J = 9.2 Hz, 1.9 Hz, 1H, H-7), 8.81 (s, 1 H, H-2), 8.79 (s, 1 H, H-7′), 8.12 (d, J = 9.4 Hz, 1 H, H-8), 7.59 (dd, J = 8.1 Hz, 1.3 Hz; 1 H, H-5′), 7.38 (d, J = 8.0 Hz, 1 H, H-4′), 5.02 (t, J = 7.1 Hz, 2 H, H-2′), 3.46 (t, J = 7.0 Hz, 2 H, H-3′); MS (APCI) 373 (100, $^{81}BrMH^+$), 371 (96, $^{79}BrMH^+$). Anal. ($C_{16}H_{11}Br_1N_4O_2$) C, H, N.

Iron powder (washed with 1 N HCl and then water and dried; 180 mg, 3.23 mmol), was added to a refluxing suspension of **38** (240 mg, 6.5 mmol) and AcOH (1.04 mL, 18 mmol) in water (75 mL) and EtOH (100 mL). After 30 min more iron powder (320 mg, 5.73 mmol) was added, and heating was continued for another 30 min. The reaction was filtered while

hot, and the solids were washed with EtOH. The combined filtrates were treated with concentrated NH_4OH (10 mL), and solids were removed by filtration. The filtrate was concentrated, and the resulting residue was purified by flash silica gel chromatography in CH_2Cl_2/MeOH (10:1) to give a solid that was freed from acetate species by dissolving it in Et_2O and washed consecutively with water, saturated aqueous $NaHCO_3$, and saturated brine. Evaporation gave 6-amino-4-[1-(6-bromoindolinyl)]quinazoline (**39**) (220 mg, 100%), which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 8.52 (s, 1 H, H-2), 7.66 (d, J = 8.9 Hz, 1 H, H), 7.31 (dd, J = 8.9 Hz, 2.4 Hz, 1 H, H-5′), 7.24 (d, J = 8.0 Hz, 1 H, H-4′), 7.18 (d, J = 1.7 Hz, 1 H, H-7′), 7.06 (dd, J = 7.8 Hz, 1.8 Hz, 1 H, H-7) 6.96 (d, J = 2.4 Hz, 1 H, H-5), 5.82 (s, 2 H, exchangeable, NH_2), 4.32 (t, J = 8.2 Hz, 2 H), 3.13 (t, J = 8.1 Hz, 2 H, H-3′); MS (APCI) 343.1 (98, $^{81}BrMH^+$), 341.1 (100, $^{79}BrMH^+$).

Acrylic acid (0.16 mL, 2.3 mmol) followed by *N*-ethyldiisopropylamine (0.40 mL, 2.3 mmol) were added to a stirred suspension of **39** (200 mg, 0.6 mmol) and EDCI·HCl (450 mg, 2.3 mmol) in DMF (1 mL) and THF (3 mL), cooled in an ice bath under nitrogen. The ice bath was removed, and the suspension was stirred at room temperature for 18 h. The resulting solution was poured into cold water, and the suspension was extracted with CH_2Cl_2 (4 × 25 mL). The extracts were dried ($MgSO_4$), concentrated under reduced pressure, and purified by flash silica gel chromatography, eluting with CH_2Cl_2/MeOH (9:1) to give **8** as a light-yellow solid (110 mg, 41%): mp 214−216 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.59 (s, 1 H, NH), 8.80 (d, J = 2.2 Hz, 1 H, H-5), 8.70 (s, 1 H, H-2), 8.03 (dd, J = 9.0 Hz, 2.3 Hz, 1 H, H-7), 7.89 (d, J = 9.2 Hz, 1 H, H-8), 7.73 (d, J = 1.7 Hz, 1 H, H-7′), 7.28 (d, J = 8.0 Hz, 1 H, H-4′), 7.16 (dd, J = 7.8 Hz, 1.8 Hz, 1 H, H-5′), 6.48 (dd, J = 17.0 Hz, 10.0 Hz, 1 H, C*H*=CH_2), 6.32 (dd, J = 16.9 Hz, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.83 (dd, J = 10.1 Hz, 1.9 Hz, 1 H, CH=C*H*$_2$), 4.51 (t, J = 8.2 Hz, 2 H, H-2′), 3.19 (t, J = 8.1 Hz, 2 H, H-3′); MS (APCI) 397.1 (96, $^{81}BrMH^+$), 395.1 (100, $^{79}BrMH^+$). Anal. ($C_{19}H_{15}Br_1N_4O_1$·0.3DMF·0.3H_2O) C, H, N.

Cell Culture. A431 human epidermoid carcinoma and MDA-MB-453 human breast carcinoma cells were obtained from the American Type Culture Collection, Rockville, MD, and maintained as monolayers in αMEM (Dulbecco's modified Eagle medium)/F12, 50:50 (Gibco/BRL), containing 10% fetal bovine serum.

Purification of Epidermal Growth Factor Receptor Tyrosine Kinase. Human EGFR tyrosine kinase was isolated from A431 human epidermoid carcinoma cells by the following method. Cells were grown in roller bottles in αMEM/F12 media (Gibco/BRL) containing 10% fetal calf serum. Approximately 10^9 cells were lysed in 2 volumes of buffer containing 20 mM 4-(2-hydroxyethyl)-1-piperazinesulfonic acid (HEPES), pH 7.4, 5 mM ethylene glycol bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1% Triton X-100, 10% glycerol, 0.1 mM sodium orthovanadate, 5 mM sodium fluoride, 4 mM pyrophosphate, 4 mM benzamide, 1 mM dithiothreitol (DTT), 80 mg/mL aprotinin, 40 mg/mL leupeptin, and 1 mM phenylmethanesulfonyl fluoride (PMSF). After centrifugation at 25000g for 10 min, the supernatant was applied to a fast Q Sepharose column (Pharmacia Biotech, Inc., Piscataway, NJ) and eluted with a linear gradient from 0.1 to 0.4 M NaCl in 50 mM Hepes, 10% glycerol, pH 7.4. Enzyme active fractions were pooled, divided into aliquots, and stored at −100 °C.

Tyrosine Kinase Assays. Enzyme assays for IC_{50} 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 mM adenosine triphosphate (ATP) containing 0.5 mCi of [^{32}P]ATP, 20 mg of poly(glutamic acid)/tyrosine (Sigma Chemical Co., St. Louis, MO), 20 ng of EGFR tyrosine kinase (for a calculated final concentration of 1.18 nM), and appropriate dilutions of inhibitor. All components except the ATP were added to the well, and the plate was incubated with shaking for 10 min at 25 °C. The reaction was started by adding [^{32}P]ATP, and the plate was 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 were then washed five times with 0.2 mL of 10% TCA, and ^{32}P incorporation was 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 μM of designated compound to be tested as an irreversible inhibitor for 1 h. One set of cells was then stimulated with 100 ng/mL EGF for 5 min, and extracts were made as described under the Western blotting procedure below. 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 were made similar to the first set of cells.

Autophosphorylation Assay. Inhibition of receptor autophosphorylation in viable cells was determined by antiphosphotyrosine Western blots. Cells were grown to 90% confluency in 6-well plates, made serum-free for 18 h, and then treated with various concentrations of compound for 1 h. The cells were then stimulated for 5 min with EGF (100 ng/mL) or heregulin (10 ng/mL), and extracts were made by lysing the monolayers in 0.2 mL of boiling Laemlli buffer (2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, and 50 mM tris(hydroxymethyl)aminomethane (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 10 mM Tris, pH 7.2, 150 mM NaCl, 0.01% azide (TNA) and blocked overnight in TNA containing 5% bovine serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody (UBI, 1 mg/mL in blocking buffer) and then washed twice in TNA, once in TNA containing 0.05% Tween-20 detergent and 0.05% nonidet P-40 detergent, and twice in TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 mCi/mL [^{125}I]protein 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 (Eastman Kodak Co., Rochester, NY) for 1−7 days. Band intensities were determined with a Molecular Dynamics laser densitometer.

In Vivo Chemotherapy. Immune-deficient mice were housed in microisolator cages within a barrier facility on a 12-h light/dark cycle and received food and water ad libitum. Animal housing was in accord with AAALAC guidelines. All experimental protocols involving animals were approved by the institutional animal care and use committee. The A431 epidermoid carcinoma and the H125 NSCLC tumor lines 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) initiated when tumors reached approximately 100−150 mg in mass and continued for the period indicated in Table 2. Whenever possible each test compound was evaluated over a range of dose levels ranging from toxic to ineffective. The doses reported in Table 2 are the maximum doses that could be administered without exceeding the LD_{10}, unless otherwise indicated. This maximum tolerated dose (MTD) allows comparisons to be made among the tested compounds at an equitoxic dose level. Doses above the 200−250 mg/kg level were not tested even if nontoxic, as compounds with even lower levels of potency would probably not be suitable for clinical development. The vehicle for compounds administered intraperitoneally was 6% dimethylacetamide and 94% 50 mM sodium lactate buffer, pH 4.0 (fine suspension). In later studies compounds were suspended in

0.5% methylcellulose in water to minimize diluent effects. 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.[37−40] A positive net cell kill indicates that the tumor burden at the end of therapy was less than at the beginning of therapy. A negative net log cell kill indicates that the tumor grew during treatment. Net cell kills near 0 indicate no tumor growth during therapy.

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