Tyrosine Kinase Inhibitors. 17. Irreversible Inhibitors of the Epidermal Growth Factor Receptor: 4-(Phenylamino)quinazoline- and 4-(Phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides Bearing Additional Solubilizing Functions

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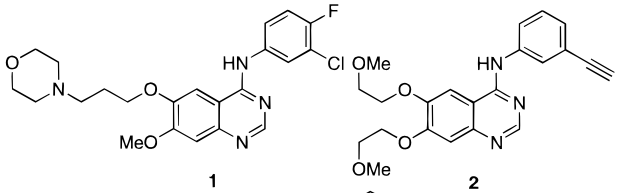
4-Anilinoquinazoline- and 4-anilinopyrido[3,2-*d*]pyrimidine-6-acrylamides substituted with solubilizing 7-alkylamine or 7-alkoxyamine side chains were prepared by reaction of the corresponding 6-amines with acrylic acid or acrylic acid anhydrides. In the pyrido[3,2-*d*]-pyrimidine series, the intermediate 6-amino-7-alkylamines were prepared from 7-bromo-6-fluoropyrido[3,2-*d*]pyrimidine via Stille coupling with the appropriate stannane under palladium-(0) catalysis. This proved a versatile method for the introduction of cationic solubilizing side chains. 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. Quinazoline analogues with 7-alkoxyamine solubilizing groups were potent irreversible inhibitors of the isolated EGFR enzyme, with $IC_{50[app]}$ values from 2 to 4 nM, and potently inhibited both EGFR and *erb*B2 autophosphorylation in cells. 7-Alkylamino- and 7-alkoxyaminopyrido[3,2-*d*]pyrimidines were also irreversible inhibitors with equal or superior potency against the isolated enzyme but were less effective in the cellular autophosphorylation assays. Both quinazoline- and pyrido[3,2-*d*]pyrimidine-6-acrylamides bound at the ATP site alkylating cysteine 773, as shown by electrospray ionization mass spectrometry, and had similar rates of absorptive and secretory transport in Caco-2 cells. A comparison of two 7-propoxymorpholide analogues showed that the pyrido[3,2-*d*]pyrimidine-6-acrylamide had greater amide instability and higher acrylamide reactivity, being converted to glutathione adducts in cells more rapidly than the corresponding quinazoline. This difference may contribute to the observed lower cellular potency of the pyrido[3,2-*d*]pyrimidine-6-acrylamides. Selected compounds showed high in vivo activity against A431 xenografts on oral dosing, with the quinazolines being superior to the pyrido[3,2-*d*]pyrimidines. Overall, the quinazolines proved superior to previous analogues in terms of aqueous solubility, potency, and in vivo antitumor activity, and one example (CI 1033) has been selected for clinical evaluation.

Introduction

Overexpression of the epidermal growth factor receptor (EGFR) tyrosine kinase is associated with poor prognosis in a significant proportion of human tumors.[1,2] Compounds that inhibit EGFR autophosphorylation and concomitantly EGF-stimulated signal transduction are potentially a new class of anticancer drugs.[3−5] The most potent and selective EGFR inhibitors reported to date are the 4-anilinoquinazolines and related 4-anilinopyrido-[*d*]pyrimidines.[6−10] These compounds bind reversibly at the ATP binding domain of EGFR, and two examples (**1**, **2**) are reported to be in clinical trial.[11,12]

In a further development of this class, we have recently reported[13,14] that acrylamide-substituted 4-anilinoquinazolines and 4-anilinopyrido[*d*]pyrimidines (e.g.



3, **4**) are potent, selective, and irreversible inhibitors of the *erb*B family of receptor tyrosine kinases. These compounds also bind in the ATP domain of the EGFR but then alkylate the enzyme at cysteine 773, irreversibly shutting down EGFR autophosphorylation.[13] They show superior in vivo activity in animal tumor models in comparison with related reversible inhibitors, par-

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ticularly by oral dosing regimens.[14] Structure−activity and molecular modeling studies have shown that positioning of the acrylamide at the 6-position is optimal for rapid irreversible inhibition (appropriate positioning of the Michael acceptor with respect to the cysteine SH).[13] Apart from this requirement, wide variation in the drug structure is tolerated, with 6-acrylamide-substituted 4-anilinoquinazolines, 4-anilinopyrido[3,4-*d*]pyrimidines, and 4-anilino[3,2-*d*]pyrimidines all being potent, selective, irreversible inhibitors of EGFR autophosphorylation.[13,14]

As part of further investigations of structure−activity relationships in this new series of irreversible EGFR inhibitors, we now report the synthesis and biological activity of a series of quinazoline-6-acrylamides (compounds **5**−**19**) and pyrido[3,2-*d*]pyrimidine-6-acrylamides (compounds **20**−**27**) substituted with solubilizing cationic side chains at positions separate from the Michael acceptor. We show that the 7-substituted compounds are superior in terms of aqueous solubility, potency, and in vivo antitumor activity to previous analogues. One example (**18**, PD 183805) has been selected for clinical evaluation (as the dihydrochloride salt, CI 1033).[15]

Chemistry

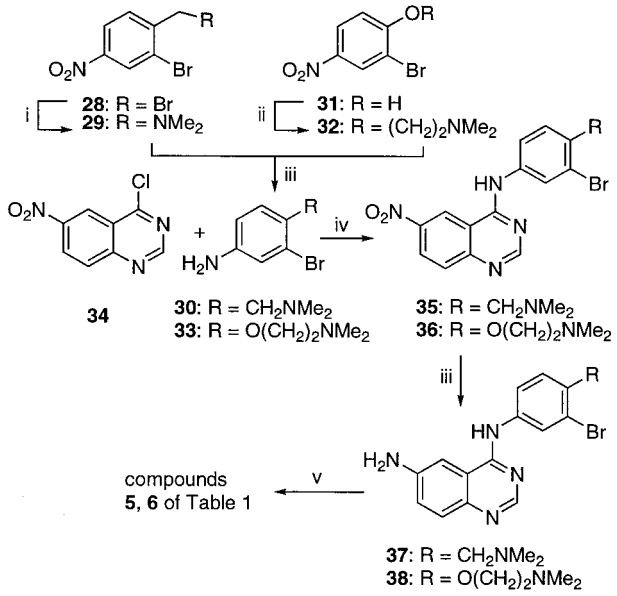
The acrylamides (**5**−**11**, **13**, **14**, **16**, **18**, **19**, **21**−**27**) of Table 1 were obtained by direct coupling of their precursor amines (**37**, **38**, **47a**−**e**, **48a**,**b**, **49b**, **50b**,**f**, **61**, **63**, **72a**−**c**, **75**, **72d,** respectively) with acrylic acid. In general 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) was employed as the coupling reagent. However acylation of amine **50f** was achieved using the mixed anhydride from acrylic acid and isobutyl chloroformate. EDCI-promoted acylation of the 6-aminopyrido[3,2-*d*]pyrimidine nucleus was exceedingly slow due to low nucleophilicity of the amines, and therefore the reactive mixed anhydride formed from acrylic acid and 2,4,6-trichlorobenzoyl chloride was employed.

The 6-aminoquinazolines **37** and **38** required first the synthesis of anilines **30** and **33** (Scheme 1). 2-Bromo-4-nitrobenzyl bromide (**28**)[16] was reacted with dimethylamine to give benzylamine **29**, which was subsequently reduced with iron dust to aniline **30**. Alkylation of 2-bromo-4-nitrophenol (**31**) with 2-dimethylaminoethyl chloride hydrochloride gave nitrobenzene **32**, which was reduced to aniline **33** by iron dust in low yield, whereas hydrogenation over Pt−C gave aniline **33** in acceptable yield (56%). Reaction of anilines **30** and **33** with 4-chloro-6-nitroquinazoline (**34**)[17] gave the 6-nitroquinazolines **35** and **36,** respectively, which underwent subsequent iron dust reduction to give the desired 6-aminoquinazolines **37** and **38**.

The 6-amino-7-alkoxyquinazolines (**47a**−**d**, **48a**,**b**, **49b**, **50b**,**f**) and the 6-amino-7-thioalkoxyquinazoline (**47e**) were each obtained from their 7-fluoro-6-nitroquinazoline precursors (**39**−**42**) by displacement of the activated fluorine with the sodium (or potassium) alkoxide (or sodium thioalkoxide) of the requisite amine side chain (Scheme 2). The sodium alkoxide was generated by reaction of the alcohol with either sodium metal or sodium hydride in THF, the latter giving a more efficient conversion and therefore better control of the number of equivalents of alkoxide employed. At least 2 equiv of alkoxide are required, with the first equivalent deprotonating the 4-anilino proton to give a characteristic dark red/purple anion in solution. However, when excess alkoxide was used in the reaction of **39** to give **43c** the only product isolated was the amine **47c** (in low yield), a result of alkoxide displacement and subsequent in situ nitro group reduction. Iron dust reduction of the 6-nitroquinazolines **43a**,**b**,**d**,**e**, **45b,** and **46b** gave the 6-aminoquinazolines **47a**,**b**,**d**,**e**, **49b**, and **50b,** respectively in excellent yield, while catalytic hydrogenation of the 6-nitroquinazolines **44a**,**b**,**f** gave the required 6-aminoquinazolines **48a**,**b** and **50f,** respectively, also in excellent yield.

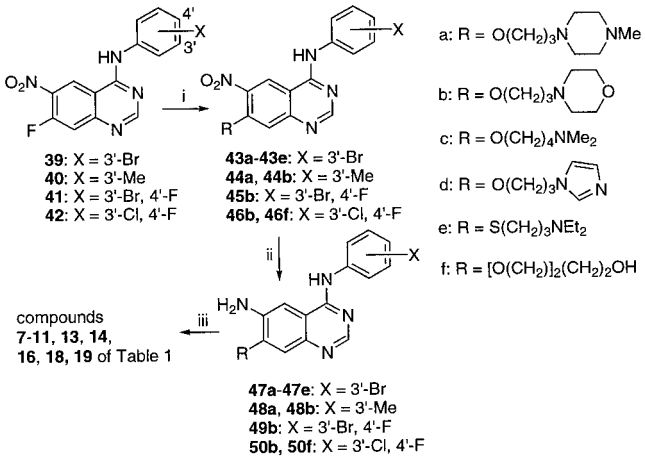
The 6-aminopyrido[3,2-*d*]pyrimidines **61** and **63** were both obtained from the key intermediate 7-bromo-6-

Scheme 1[a]



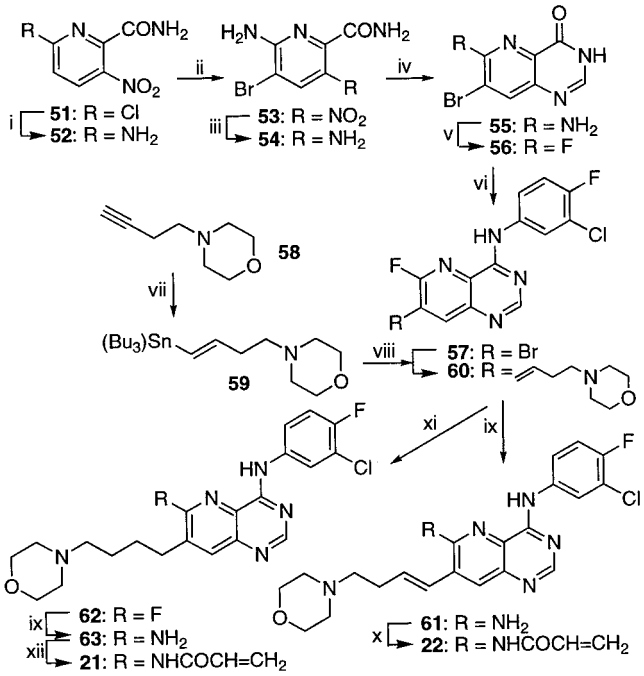
[a] (i) MeOH/Me_2NH/20 °C/1 h; (ii) $Cl(CH_2)_2NMe{\cdot}HCl/K_2CO_3$/DMF/100 °C/4 h; (iii) Fe/AcOH/EtOH/H_2O/reflux/20 min or H_2/Pt-C (for **32**); (iv) *i*-PrOH/CH_2Cl_2/Et_3N/reflux/3 h; (v) $CH_2{=}CHCO_2H$/EDCI·HCl/pyridine/DMA/20 °C/2 h.

Scheme 2[a]



[a] (i) RO^-Na^+/THF/reflux/18−24 h or RS^-Na^+/DMSO/65 °C (for **39**−**43e**) or RO^-K^+/DMSO/25 °C (for **42**−**46f**); (ii) Fe/AcOH/EtOH/H_2O/reflux/20 min or H_2/Pd−C/MeOH:EtOAc (2:1) (for X = 3′-Me) or H_2/Raney Ni/THF (for **46f**); (iii) $CH_2{=}CHCO_2H$/EDCI·HCl/pyridine or Et_3N/DMA or DMF/2 h or (for **50f**) $CH_2{=}CHCO_2H$/*i*-BuOCOCl/Et_3N/THF.

Scheme 3[a]

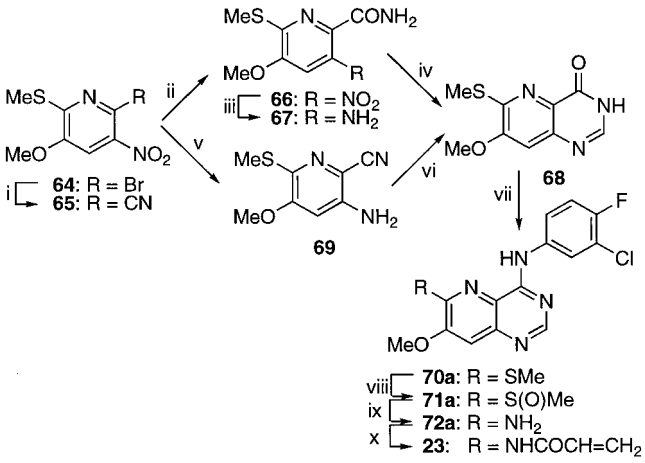


[a] (i) NH_4OH/EtOH/100 °C/20 h (pressure vessel); (ii) Br_2/AcOH/HOAc/20 °C/90 min; (iii) Fe/AcOH/EtOH/H_2O/reflux/10 min; (iv) $(EtO)_3CH$/reflux/3 h; (v) $HBF_4/NaNO_2$/−20 °C/18 h; (vi) $POCl_3$/reflux/110 min, then 3-chloro-4-fluoroaniline/*i*-PrOH/60 °C/30 min; (vii) AIBN/$(Bu)_3SnH$/90 °C/16 h; (viii) $(Ph_3P)_4Pd$/DMF/100 °C/75 min; (ix) NH_3(g)/*i*-PrOH/100 °C/24 h (pressure vessel); (x) CH_2═$CHCO_2H$/2,4,6-triClPhCOCl/Et_3N/THF/0 °C/15 min, then **61**/DMAP/THF/0 °C/15 min to rt/2 h; (xi) PtO_2/H_2/THF/MeOH/20 °C/18 h; (xii) HOBT/Et_3N/CH_2═$CHCO_2H$/EDCI·HCl/DMA/THF/20 °C/6 weeks.

fluoropyrido[3,2-*d*]pyrimidine (**57**) (Scheme 3). This intermediate is synthesized in good overall yield from chloropyridine **51**.[9] Initial reaction with ammonia introduces the amino functionality at the 2-position of pyridine **52**, which was required for the activation and directing of subsequent bromination at the adjacent 3-position, to give the bromopyridine **53**. Iron dust reduction of **53** gave diamine **54** which was converted to the pyrido[3,2-*d*]pyrimidone **55** in refluxing triethyl orthoformate. At this stage the 6-amino group of **55** is essentially "protected" by diazotization to fluorine, to give the 6-bromo-7-fluoropyrido[3,2-*d*]pyrimidone (**56**) (at a later stage this activated fluorine is readily displaced by ammonia to regenerate the required amine functionality at this position). Chlorination of the pyrimidone **56** in $POCl_3$ and displacement with 3-chloro-4-fluoroaniline gave the required intermediate 7-bromo-6-fluoropyrido[3,2-*d*]pyrimidine (**57**).

Model studies showed that Stille coupling of **57** with tetravinyltin and allyltributylstannane gave the respective 7-vinyl and 7-allyl derivatives of **57** in good yield (data not shown), with the expected complete selectivity for bromine over chlorine and fluorine. Therefore it was decided to introduce the required alkylmorpholine side chain via Stille coupling of the morpholinostannane **59**. Stannane **59** was synthesized in good yield from 3-butyn-1-ol by mesylation and displacement with morpholine to give butyne (**58**) which underwent AIBN-initiated radical hydrostannylation with tributyltin hydride to give predominantly the *E*-stannane **59** which could be further purified by chromatography. Stille coupling of

Scheme 4[a]



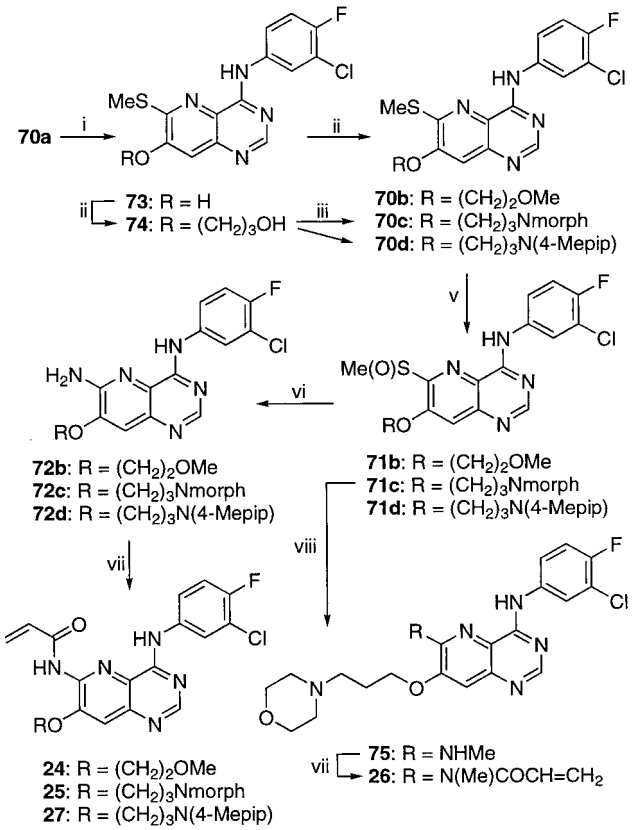
[a] (i) CuCN/NMP; (ii) 90% H_2SO_4; (iii) H_2/Pd−C; (iv) $(EtO)_3CH$/reflux/8 h; (v) Fe/AcOH/EtOH/H_2O/reflux/20 min; (vi) HCO_2H/H_2SO_4/reflux/16 h; (vii) $POCl_3$, then $ArNH_2$; (viii) *m*-CPBA/CH_2Cl_2/20 °C/ 1 h; (ix) NH_3(g)/*i*-PrOH/100 °C/20 h (pressure vessel); (x) CH_2═$CHCO_2H$/Et_3N, then 2,4,6-triClPhCOCl, then **72a**/DMAP/THF/0 °C/15 min to rt/2 h.

stannane **59** with the 7-bromopyrido[3,2-*d*]pyrimidine (**57**) under palladium(0) catalysis gave the morpholinylbutenylpyrido[3,2-*d*]pyrimidine **60** in high yield (72%). This Stille coupling approach to the introduction of cationic solubilizing side chains in medicinal chemistry is novel to our knowledge and may prove to be a versatile method for analogue generation in the future, particularly lending itself to combinatorial methods. Direct displacement of the fluorine in pyridopyrimidine **60** by ammonia gave the required amine **61**, while hydrogenation of **60** over PtO_2 to give fluoro derivative **62** and then ammonia treatment gave amine **63**.

Synthesis of the 6-amino-7-alkoxypyrido[3,2-*d*]pyrimidines (**72a**−**d**) and the 6-methylamine (**75**) required first the synthesis of the key intermediate 7-methoxy-6-(methylthio)pyrido[3,2-*d*]pyrimidine (**70a**) (Scheme 4). This pivotal intermediate was obtained in four steps from 2-bromo-5-methoxy-6-(methylthio)-3-nitropyridine[18] (**64**) in good overall yield. Initially direct displacement of the activated bromine with cyanide anion to give the nitrile **65** was followed by hydrolysis of the cyano moiety to give the carboxamide **66** which was reduced with iron dust to amine **67** and subsequently reacted with triethyl orthoformate to give pyrimidone **68**. However low yields for the acid hydrolysis of nitrile **65** could not be overcome, so an alternate route was used. This involved iron dust reduction of **65** and then direct cyclization[19] of the resulting aminopyridonitrile **69** in 88% formic acid containing a small amount of concentrated H_2SO_4 and gave the pyrimidone **68** in excellent yield. Chlorination in $POCl_3$ and reaction with 3-chloro-4-fluoroaniline gave the required intermediate **70a**. Oxidation of the methylthio derivative **70a** by *m*-CPBA to the methyl sulfoxide **71a**, followed by reaction with ammonia, gave the 6-amino-7-methoxypyrido[3,2-*d*]pyrimidine (**72a**).

Selective cleavage of the methyl ether of **70a** in the presence of the methylthio moiety was achieved with pyridine hydrochloride to give the phenol **73** (Scheme 5), which was then alkylated with either 3-bromopropanol or 2-bromoethylmethyl ether to give the alkoxy derivatives **74** and **70b,** respectively. Mesylation of **74** and reaction with morpholine or 4-methylpiperazine

Scheme 5[a]



[a] (i) Pyridine·HCl/200−210 °C/8 min; (ii) $Br(CH_2)_3OH$ or $Br(CH_2)_2OMe/K_2CO_3$/THF/DMF/reflux/1−2 h; (iii) Et_3N/MsCl/THF/DMF/0 °C/1 h, then morpholine or 4-Me-piperazine/reflux/4−5 days; (v) *m*-CPBA/CH_2Cl_2/rt/1 h or $NaIO_4$/1 N HCl/4 °C (for **70c**,**d**); (vi) NH_3(g)/*i*-PrOH/100 °C/20 h (pressure vessel); (vii) CH_2=$CHCO_2H$/Et_3N, then 2,4,6-triClPhCOCl, then **72b**−**d**/DMAP/THF/0 °C/15 min to rt/2 h; (viii) $MeNH_2$/water/DMSO/110 °C/18 h (pressure vessel).

provided the morpholinopropyloxy and 4-methylpiperazinylpropyloxy derivatives **70c**,**d,** respectively. Oxidation of the 7-alkoxy-6-methylthiopyrido[3,2-*d*]pyrimidine (**70b**) with *m*-CPBA gave the methylsulfinyl compound **71b**, while sodium periodate oxidation of methylthio derivatives **70c**,**d** gave their respective methylsulfinyl derivatives **71c**,**d**. Tertiary amine *N*-oxide formation was prevented during the latter oxidation of amines **70c**,**d** by performing the reaction in 1 N HCl. Reaction of **71b**−**d** with ammonia provided the 6-amino derivatives **72b**−**d**, while reaction of methyl sulfoxide **71c** with methylamine provided the 6-methylamino derivative **75**

Results and Discussion

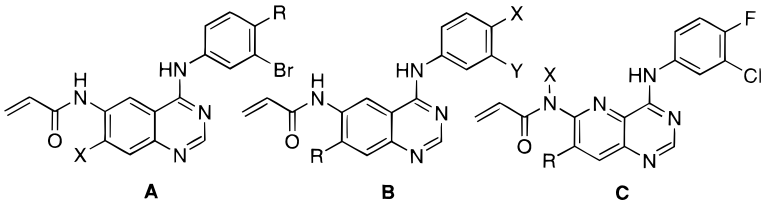
The structures and physicochemical properties of the acrylamides studied are listed in Table 1, together with their potencies ($IC_{50[app]}$ in nM) for inhibition of phosphorylation of a random glutamic acid/tyrosine copolymer substrate by isolated EGFR enzyme, inhibition of EGF-stimulated autophosphorylation of EGFR in A431 cells, and (for some examples) inhibition of heregulin-stimulated autophosphorylation of erbB2 in MDA-MB 453 cells.[10] The type of inhibition of the isolated EGFR enzyme is also listed. Irreversible inhibition is defined[14] as 80% or greater inhibition after a 10-min exposure to drug followed by drug washout and restimulation by EGF 8 h later. Drugs that produced 20−80% inhibition were designated as partially irreversible (although in reality they 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] 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.

Cationic Side Chains at the 4′-Position of the Aniline. We have previously reported[14] that in the 6-acrylamido-4-anilinoquinazoline series there is some bulk tolerance at the 4′-position of the aniline ring, with both phenyloxy and benzyloxy analogues retaining potent (IC_{50}s 4.8 and 4.2 nM, respectively) and fully irreversible inhibition of cellular autophosphorylation. Substitution at this position with relatively sterically nondemanding cationic side chains was therefore evaluated in a search for more soluble irreversible inhibitors. A 3′-bromo substituent, known to enhance the EGFR binding of reversible inhibitors,[7] was also added. Both the 3′-bromo-4′-(*N*,*N*-dimethylaminomethyl) and 3′-bromo-4′-(*N*,*N*-dimethylaminoethoxy) analogues (**5** and **6**) showed improved aqueous solubility but were not irreversible inhibitors. They also showed a large reduction in potency in both the isolated enzyme (32−54-fold) and cellular (155−2850-fold) assays, compared to the unsubstituted quinazoline (**3**). There thus appears to be no tolerance at the 4′-position of the aniline ring for cationic side chains, with such substitutions resulting in a loss of binding affinity in the ATP binding domain of EGFR and a subsequent loss of delivery of the acrylamide moiety to the key cysteine of the active site.

Cationic Side Chains at the 7-Position of the Quinazoline. Both molecular modeling[20] and structure−activity relationship studies[21] in the quinazoline and pyrido[*d*]pyrimidine series of reversible EGFR inhibitors have shown that there is tolerance for substitution at the 7-position with soluble cationic side chains. Compounds **7**−**19** explore the concept of substituting the optimal 6-acrylamide series of 4-anilinoquinazolines at the available 7-position with a series of solubilizing cationic side chains. Compounds **7**−**11**, with a fixed 3′-Br substituent, employ a range of side chains of varying pK_a, found to be acceptable in previous studies of related reversible analogues,[21] where they provided solubilities of >40 mM. The *N*-methylpiperazinylpropoxy (**7**), morpholinylpropoxy (**8**), and *N*,*N*-dimethylaminobutoxy (**9**) showed comparable activity ($IC_{50[app]}$s 2−4 nM) in both the isolated enzyme and cellular autophosphorylation assays, with the 1-imidazolylpropoxy (**10**) being about 3−6-fold less potent in the autophosphorylation assay. All were irreversible inhibitors. In contrast, the $S(CH_2)_3NEt_2$ analogue **11** was only partially irreversible and much less effective in the autophosphorylation assay.

The *N*-methylpiperazinylpropoxy and morpholinylpropoxy side chains were also employed in the 3′-Me series (compounds **13** and **14**), since the parent compound (**12**) had been shown previously[14] to be a potent irreversible inhibitor. Both compounds retained their

Table 1. Kinase Inhibition by Soluble 4-(Phenylamino)quinazoline- and 4-(Phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides



						EGFR			*erb*B2
no.	Fm	R	X	Y	mp (°C)	enz[a]	auto[b]	type[c]	auto[d]
3	A	H	H		ref 14	0.70	2.7	irrev	5.7
5	A	CH_2NMe_2	H		206	45	416	rev	
6	A	$OCH_2CH_2NMe_2$	H		181−184.5	27	7693	rev	
7	B	$O(CH_2)_3$4-Mepip	H	Br	105−107	1.7	3	irrev	
8	B	$O(CH_2)_3$morph	H	Br	170−172	3.6	5.3	irrev	6.4
9	B	$O(CH_2)_4NMe_2$	H	Br	112−115	3.9	7.9	irrev	
10	B	$O(CH_2)_3$imidazoyl	H	Br	235−237	3.0	21	irrev	
11	B	$S(CH_2)_3NEt_2$	H	Br	77−79	0.78	196	partial	400
12	B	H	H	CH_3	ref 14	0.42	4.7	irrev	22
13	B	$O(CH_2)_3$4-Mepip	H	CH_3	60−66	2.0	18	irrev	
14	B	$O(CH_2)_3$morph	H	CH_3	69−72	1.5	27	irrev	
15	B	H	F	Br	ref 14	0.69	2.7	irrev	7.3
16	B	$O(CH_2)_3$morph	F	Br	171−173	1.8	4.7	irrev	8.2
17	B	H	F	Cl	ref 14	0.75	3.1	irrev	4.3
18	B	$O(CH_2)_3$morph	F	Cl	188−190	1.5	7.4	irrev	9.0
19	B	$[O(CH_2)_2]_2(CH_2)_2OH$	F	Cl	131−132	1.7	ND	ND	
20	C	H	H		ref 14	0.75	18	irrev	12
21	C	$CH{=}CH(CH_2)_2$morph	H		190−193	0.16	119	irrev	12
22	C	$(CH_2)_4$morph	H		180−182	2.7	5100	irrev	>5000
23	C	OMe	H		226−228	0.95	291	partial	125
24	C	$O(CH_2)_2OMe$	H		205−206	0.97	377	partial	
25	C	$O(CH_2)_3$morph	H		185−186	1.5	434	irrev	189
26	C	$O(CH_2)_3$morph	Me		176−178	20	88	partial	>200
27	C	$O(CH_2)_3$4-Mepip	H		166−168	6.6	264	partial	182

[a] Concentration ($IC_{50[app]}$ in nM) 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 (IC_{50} in nM) to inhibit by 50% the phosphorylation 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] IC_{50} values (nM) for inhibition of autophosphorylation of *erb*B2 (in MDA-MB 453 cells) in culture. Values are the average of at least two experiments.

ability to irreversibly inhibit EGFR, and while they were as potent as the 3′-Br derivatives in the isolated enzyme assay, they were somewhat less potent at inhibiting cellular autophosphorylation (IC_{50}s 18 and 27 nM, respectively).

Two 4′-F analogues with the morpholinylpropoxy solubilizing group (**16** and **18**) were also studied (using respectively 3′-Br and 3′-Cl binding enhancers), since work by others[22] with reversible dianilinophthalimide EGFR inhibitors has shown that a *p*-F substituent provides longer in vivo half-lives, possibly by blocking metabolism. Both of these compounds had similar potencies to their nonsolubilized analogues (**15** and **17,** respectively) and also compared favorably to the corresponding nonfluorinated analogue **8**, suggesting that better metabolic stability can be provided without compromising inhibitory potency. Compound **19**, which explored the use of a neutral oxygenated solubilizing function, showed reasonably potent enzyme inhibitory properties but no distinct solubility advantage and was therefore not studied further.

The quinazolines **8**, **16,** and **18,** bearing cationic side chains, show excellent potency for inhibition of *erb*B2 autophosphorylation in MDA-MB 453 cells (IC_{50}s 6.4, 8.2, and 9.0 nM, respectively). We have previously reported[13] an acrylamide-bearing quinazoline capable of covalently modifying both EGFR and *erb*B2, both of which have the key cysteine 773 at the entrance of their ATP binding domain. It therefore seems likely that the excellent activity shown by quinazolines **8**, **16,** and **18** against *erb*B2 is due also to irreversible inhibition of this enzyme. These compounds therefore represent a novel class of potent, soluble, irreversible inhibitors of the larger EGFR family of tyrosine kinases.

Cationic Side Chains at the 7-Position of the Pyrido[3,2-*d*]pyrimidine. Because the pyrido[3,2-*d*]-pyrimidines are also potent reversible inhibitors of EGFR,[9] it was of interest to see whether 6-acrylamide analogues of this additional series were also irreversible inhibitors. The pyrido[3,2-*d*]pyrimidine **20** was therefore prepared[14] and shown to be an irreversible inhibitor, with similar potency against the isolated enzyme to the corresponding quinazoline (**17**) ($IC_{50[app]}$s both 0.75 nM), but with slightly lower potency in the autophosphorylation assay (IC_{50}s 18 and 3.1 nM, respectively). The pyrido[3,2-*d*]pyrimidine chromophore also provided an opportunity to introduce solubilizing side chains at the 7-position while retaining the optimally positioned 6-acrylamide,[13,14] and a small series of analogues of **20** with solubilizing 7-substituents were prepared. The "carbon-linked" morpholinylbutyl derivative (**22**) showed good activity against the isolated enzyme ($IC_{50[app]}$ 2.8 nM) but a large loss of potency in the cellular autophosphorylation assays against EGFR and *erb*B2 (IC_{50}s 5100

and >5000 nM, respectively). However, the corresponding unsaturated derivative (**21**) was the most potent inhibitor of the isolated EGFR enzyme in the series, with an $IC_{50[app]}$ of 0.16 nM, possibly suggesting an advantage in terms of potency when the 7-side chain is conformationally restrained close to the chromophore ring system. Derivative **21** was less active in the EGFR autophosphorylation assay in A431 cells (although not to the same extent as derivative **22**), with an IC_{50} of 119 nM, although it had high potency (IC_{50} 12 nM) in the cellular *erb*B2 autophosphorylation assay (in MDA-MB 453 cells), showing the largest differential toward *erb*B2 of all the compounds evaluated. The general loss of potency for the "carbon-linked" solubilized pyrido[3,2-*d*]pyrimidines in the cellular assays, except for the *erb*B2 potency of compound **21**, suggests these compounds may suffer from metabolism, transport, permeability, or stability problems in a cell-line-specific manner.

One possibility was that the "carbon-linked" soluble side chains were undergoing cellular metabolism at the benzylic position. This was consistent with the saturated derivative (**22**) being less potent than the unsaturated derivative (**21**). Therefore a series of "oxygen-linked" direct pyrido[3,2-*d*]pyrimidine analogues of quinazoline **18** were prepared. However, while these (**23**−**27**) were generally also potent inhibitors of the isolated EGFR enzyme, they were much less effective than the quinazolines in inhibiting EGFR and *erb*B2 autophosphorylation in cells and were generally classed as only partially irreversible in the 8-h washout assay.

Comparisons of Quinazoline and Pyrido[3,2-*d*]-pyrimidine Analogues. The reasons for the loss of cellular activity of the pyrido[3,2-*d*]pyrimidines compared with the corresponding quinazolines were sought in a number of comparative studies. The binding stoichiometry and the site(s) of interaction between EGF receptor tyrosine kinase and the morpholinylbutylpyrido[3,2-*d*]pyrimidine **22** was determined through the use of electrospray ionization mass spectrometry (ESI-MS),[23] along with that of the quinazoline **18** as a positive control.[13] The molecular weight of the EGF receptor complexed with the compounds, as measured by ESI-MS, was approximately 485 Da higher than the apoprotein; the mass difference is consistent with a covalent 1:1 protein:drug complex.[13] A small amount (10−20% relative abundance) of 1:2 protein:drug complex was also observed. Trypsin digestion of the drug-bound protein and analyses by LC-ESI tandem mass spectrometry (MS/MS) identified cysteine 773 as the predominant site of interaction for both compounds, suggesting that a change in the target site was not the reason for the different activities.

The absorptive and secretory transport of the quinazoline **18** and the pyrido[3,2-*d*]pyrimidine **25** were compared in Caco-2 cells[24] (Table 2). Activity of P-gp in the Caco-2 cells was confirmed by substantially greater basolateral-to-apical (B→A) versus apical-to-basolateral (A→B) permeability of [^{3}H]vinblastine, a known substrate of P-gp[25,26] (Table 2). Compounds **18** and **25** both showed high permeability in Caco-2 cells (greater than the experimentally determined permeability of [^{3}H]-metoprolol, which is 90−95% absorbed in humans). The B→A/A→B ratios of 1.2 and 1.36 for **18** and **25**, respectively, suggest that efflux is not a concern with either

Table 2. Absorptive and Secretory Transport of **18** and **25** in Caco-2 Cells in Comparison to Metoprolol (90−95% absorbed) and Vinblastine (P-gp substrate)

compd	permeability × 10^6 (cm/s)[a] absorption	secretion	permeability limited?	efflux limited?
18	49.3 ± 8.92	59.1 ± 4.81	no	no
25	59.6 ± 7.38	81.2 ± 4.80	no	no
metoprolol	21.4 ± 3.26	38.2 ± 7.47	no	no
vinblastine	1.54 ± 1.91	12.5 ± 4.47	yes	yes

[a] Data are reported as means ± SD from $n = 3$. Values for [^{14}C]mannitol (leakage marker) ranged from 0.74 to 0.761 × 10^{-6} cm/s for A→B studies and from 1.13 to 1.99 × 10^{-6} cm/s for B→A studies.

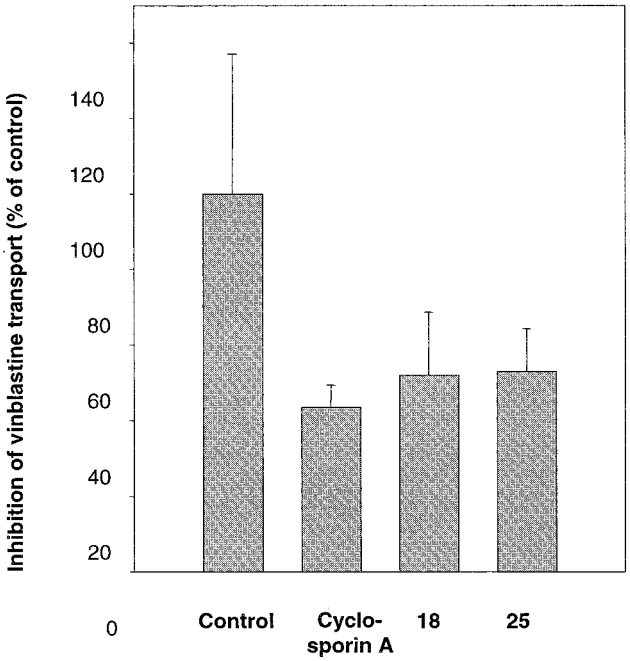


Figure 1. Inhibition of the secretory transport of vinblastine in Caco-2 cells by compounds **18** and **25**, in comparison to the known inhibitor cyclosporin A (mean ± SD, $n = 3$).

of these compounds. The effect of **18** and **25** on inhibition of the secretory transport of vinblastine was also carried out in comparison with the known inhibitor cyclosporin A (Figure 1). Even though none of the inhibitory results were significantly different from the control, both compounds are likely inhibitors of P-gp, as evidenced by their similar effect on vinblastine efflux as compared with cyclosporin A.[27,28]

The structural similarity and above similar biochemical properties of **25** compared with **18** (which showed potent inhibition of autophosphorylation) make the poor cellular activity of the former difficult to understand. The Caco-2 studies show that **25** has cellular permeability and efflux characteristics similar to those of **18**.

Another possibility for the poor cellular activity of the pyrido[3,2-*d*]pyrimidines (e.g., **25** versus **18**) was lower stability of the acrylamide moiety in the former. Accelerated cleavage of the acrylamide group of **25**, either by cellular amidases or a hydrolytic mechanism to give the 6-amino derivative **72c,** may lower its activity (**72c** has low potency in autophosphorylation assays, with an IC_{50} for inhibition of heregulin-stimulated *erb*B2 autophosphorylation in MDA-MB 453 cells of 430 nM). This was explored by comparative stability studies of **25** and **18**. Solutions of these compounds in phosphate buffers

Table 3. In Vivo Antitumor Activities of Selected Soluble 4-Anilinoquinazoline- and 4-Anilinopyrido[3,2-*d*]pyrimidine-6-acrylamides in A431 Xenografts

no.	dose[a] (mg/kg)	schedule[b]	weight change (g)	T/C (%) last therapy day[c]	T−C[d] (days)
8	10	ip, b.i.d., days 13−26	+	18	21.5
	60	po, days 10−24	−1.6	0	29.8
18	18	po, days 10−24	−1.0	0	41.3
	5[e]	po, days 10−24	−0.4	4	53.2
25	20[e]	po, days 15−19, 22−26, 29−33	−0.5	69	9.3

[a] Maximum tolerated dose (not exceeding LD_{10}). [b] Compounds were administered in solution as the isethionate salts (**8** and **18**) or as a solution in lactate buffer (**25**), either intraperitoneally or orally on the indicated schedules. (Tumors were implanted sc into the right axilla of mice on day 0.) [c] Ratio of median treated tumor mass/median control tumor mass × 100. [d] The difference in days for treated (T) and control (C) tumors to reach 750 mg. [e] Not a maximum tolerated dose.

at pH 2.6, 6.67, and 10.75 were kept at 37 °C and monitored by HPLC for 24 h for formation of the corresponding 6-amine hydrolysis products (**72c** and **50b,** respectively). The quinazoline **18** was stable at all pH levels. In contrast, while the pyrido[3,2-*d*]pyrimidine **25** was stable at pH 2.6 and 6.67, it was quite unstable at pH 10.75, hydrolyzing to the corresponding amine **72c** with a half-life of about 2.5 h. Further support for this hypothesis was provided by the *N*-methylacrylamide **26** (the direct analogue of **25**). While **26** showed reduced potency in the isolated enzyme assay ($IC_{50[app]}$ 20 nM) and only partially irreversible inhibition (consistent with a reduced binding affinity in the ATP binding domain due to *N*-methylation), it showed improved cellular potency against EGFR autophosphorylation (IC_{50} 88 nM compared to **25** with IC_{50} 434 nM). This is consistent with higher metabolic stability. Finally, mass spectral studies of the metabolism of the two compounds in A431 cells in culture showed that **25** was converted much more rapidly than **18** to the corrresponding glutathione adducts. This higher apparent reactivity of the acrylamide in the pyrido[3,2-*d*]-pyrimidine analogue may also contribute to the lower effectiveness of this compound in cells.

In Vivo Activity. The quinazolines **8** and **18** and the pyrido[3,2-*d*]pyrimidine **25** were evaluated against A431 xenografts in mice, and the results are given in Table 3. Both **8** and **18** showed impressive activity when dosed orally for 14 days, but the derivative **18** was much more potent (optimal dose 5 mg/kg/day) compared to the other analogues. The pyrido[3,2-*d*]pyrimidine **25** was only minimally effective, indicating a very low dose potency for this compared to both the other derivatives tested even though it was equally soluble. The essentially equivalent antitumor activity for **18** at the two dose levels shown in Table 3 suggests that this compound might have a good therapeutic index. Weight loss, as an indicator of compound-induced toxicity, was minimal in the experimental animals, being less than 10% at tolerated dose levels.

Conclusions

New and feasible synthetic routes to 4-anilinopyrido-[3,2-*d*]pyrimidine-6-acrylamides bearing solubilizing 7-alkylamino and 7-alkoxyamino side chains have been developed. Stille coupling of stannanes with the 7-bromopyrido[3,2-*d*]pyrimidine (**57**) under palladium(0) catalysis was a facile way of introducing the 7-alkylamino side chains and could be more broadly used in medicinal chemistry. The solubilizing side chains do not alter the previously determined binding mode of these compounds to the EGFR enzyme: binding at the ATP site and alkylating cysteine 773. They were potent and irreversible inhibitors of the isolated enzyme, and the quinazolines were also potent inhibitors of both EGFR and *erb*B2 autophosphorylation in cells, although the corresponding pyrido[3,2-*d*]pyrimidines (both 7-alkylamine and 7-alkoxyamine substituted) were less effective in the cellular assays. The reason for this is most likely due to differences in the chemistry of the acrylamide side chain, which in the pyrido[3,2-*d*]pyrimidines was less stable to both chemical hydrolysis at high pH to the amine (this may be a marker for more rapid hydrolysis by amidases) and cellular metabolism to the gluthathione conjugate.

The quinazoline propoxymorpholide **18** showed superior in vivo antitumor activity to previous nonsolubilized analogues, giving growth delays in A431 xenografts exceeding 50 days following oral administration. This compound (as the dihydrochloride salt, CI 1033) has been selected for clinical evaluation.[15]

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 on either a Varian VG 7070 spectrometer at nominal 5000 resolution or a Finnigan MAT 900Q spectrometer.

***N*-[4-[*N*-[3-Bromo-4-(dimethylaminomethyl)phenyl]amino]quinazolin-6-yl]acrylamide (5). Example of the Method of Scheme 1.** To a solution of 2-bromo-4-nitrobenzyl bromide[16] (**28**) (7.00 g, 23.7 mmol) in MeOH (100 mL) was added excess dimethylamine (15 mL, 40% solution in water). After 1 h at room temperature the reaction mixture was concentrated under reduced pressure, diluted with water and extracted with EtOAc. The combined organic extracts were then extracted with 1 N HCl which was then basified with concentrated NH_4-OH and extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure before being chromatographed on silica gel eluting with EtOAc to give 2-bromo-4-nitro-*N*,*N*-dimethylbenzylamine (**29**) as an oil (5.0 g, 62%): ^{1}H NMR [$(CD_3)_2SO$] δ 8.40 (d, J = 2.4 Hz, 1 H, H-3), 8.24 (dd, J = 8.5, 2.5 Hz, 1 H, H-5), 7.75 (d, J = 8.5 Hz, 1 H, H-6), 3.56 (s, 2 H, $ArCH_2N$), 2.24 (s, 6 H, $N(CH_3)_2$). HRMS (EI) Calculated for $C_9H_{11}{}^{79}BrN_2O_2$: 258.0004. Found: 258.0003.

Iron powder (4.00 mol equiv, 5.46 g; freshly washed with 1 N HCl followed by distilled water) was added in portions to a refluxing solution of **29** (6.00 g, 24.5 mmol) in EtOH/H_2O (2:1, 180 mL) containing glacial AcOH (6.0 mL). The resulting suspension was heated at reflux with vigorous stirring for 20 min, then cooled, basified with concentrated NH_4OH and filtered through a pad of Celite. The Celite was washed with EtOH, and the combined filtrate was concentrated under reduced pressure, diluted with water and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and chromatographed on grade III alumina, eluting with EtOAc/hexane (1:2), to give 4-amino-2-bromo-*N*,*N*-dimethylbenzylamine (**30**) (5.03 g, 96%), which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 7.01 (d, J = 8.2 Hz, 1 H, H-6), 6.77 (d, J = 2.2 Hz, 1 H, H-3), 6.51 (dd, J = 8.2, 2.2 Hz, 1 H, H-5), 5.26 (s, 2 H, NH_2), 3.26 (s, 2 H, $ArCH_2N$), 2.12 (s, 6 H, $N(CH_3)_2$).

To a solution of 2-bromo-4-nitrophenol (**31**) (2.50 g, 12.3 mmol) in DMF (30 mL) were added 2-dimethylaminoethyl chloride hydrochloride (2.65 g, 18.4 mmol) and K_2CO_3 (5.08 g, 36.8 mmol). The resulting suspension was heated at 100 °C with stirring for 2 h before further 2-dimethylaminoethyl chloride hydrochloride (1.0 g) was added. After a further 2 h the DMF was removed under reduced pressure, the residue was diluted with NH_4OH and extracted with EtOAc. The combined organic extracts were then dried (Na_2SO_4) and concentrated under reduced pressure before being chromatographed on grade III alumina, eluting with EtOAc/hexane (1:2), to give 2-bromo-1-[2-(dimethylamino)ethoxy]-4-nitrobenzene (**32**) (0.96 g, 27%) as a glassy solid: ^{1}H NMR [$(CD_3)_2SO$] δ 8.42 (d, J = 2.7 Hz, 1 H, H-3), 8.26 (dd, J = 9.2, 2.7 Hz, 1 H, H-5), 7.36 (d, J = 9.2 Hz, 1 H, H-6), 4.30 (t, J = 5.6 Hz, 2 H, OCH_2CH_2N), 2.72 (t, J = 5.6 Hz, 2 H, OCH_2CH_2N), 2.25 (s, 6 H, $N(CH_3)_2$). HRMS (EI) Calculated for $C_{10}H_{13}{}^{79}BrN_2O_3$: 288.01095. Found: 288.01097.

A solution of **32** (0.96 g, 3.39 mmol) in EtOAc/MeOH (60 mL, 5:1) was hydrogenated at 60 psi over Pt−C for 25 min before being filtered through Celite, concentrated and chromatographed on grade III alumina, eluting with EtOAc/hexane (1:1), to give 4-amino-2-bromo-1-[2-(dimethylamino)ethoxy]-benzene (**33**) (0.49 g, 56%), which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ ^{1}H NMR [$(CD_3)_2SO$] δ 6.84 (d, J = 8.7 Hz, 1 H, H-6), 6.79 (d, J = 2.6 Hz, 1 H, H-3), 6.51 (dd, J = 8.7, 2.6 Hz, 1 H, H-5), 4.87 (s, 2 H, NH_2), 3.93 (t, J = 6.0 Hz, 2 H, OCH_2-CH_2N), 2.59 (t, J = 6.0 Hz, 2 H, OCH_2CH_2N), 2.23 (s, 6 H, $N(CH_3)_2$).

A suspension of 6-nitroquinazolone (3.50 g, 18.5 mmol) in neat $SOCl_2$ (30 mL) containing 2 drops of DMF was refluxed for 3 h until it became clear. The excess $SOCl_2$ was removed under reduced pressure, and dry benzene was added and then evaporated under reduced pressure to remove all traces of $SOCl_2$. The resulting crude 4-chloro-6-nitroquinazoline[17] (**34**) was dissolved in dry CH_2Cl_2 (50 mL) and washed twice with saturated Na_2CO_3, and this solution was then added to a solution of 4-amino-2-bromo-*N*,*N*-dimethylbenzylamine (**30**) (20.3 mmol, 4.64 g) in *i*-PrOH (60 mL) containing Et_3N (excess, 7.0 mL). The resulting reaction mixture was heated at reflux for 3 h and then concentrated under reduced pressure, diluted with water and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (2:9:9) to give 4-[*N*-[3-bromo-4-(dimethylaminomethyl)phenyl]-amino]-6-nitroquinazoline (**35**) (0.62 g, 8%): mp (CH_2Cl_2) 198−200 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.47 (br s, 1 H, NH), 9.66 (d, J = 2.5 Hz, 1 H, H-5), 8.77 (s, 1 H, H-2), 8.57 (dd, J = 9.2, 2.5 Hz, 1 H, H-7), 8.21 (d, J = 2.0 Hz, 1 H, H-2′), 7.95 (d, J = 9.2 Hz, 1 H, H-8), 7.91 (dd, J = 8.5, 2.0 Hz, 1 H, H-6′), 7.49 (d, J = 8.5 Hz, 1 H, H-5′), 3.46 (s, 2 H, $CH_2N(CH_3)_2$), 2.22 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{17}H_{16}BrN_5O_2\cdot1.5H_2O$) C, H, N.

Iron dust reduction of **35** (0.52 g, 1.29 mmol) in EtOH/H_2O (2:1, 50 mL) containing glacial AcOH (0.7 mL) as described above gave, after chromatography on grade III alumina, eluting with EtOAc to MeOH/EtOAc (5:95), 6-amino-4-[*N*-[3-bromo-4-(dimethylaminomethyl)phenyl]amino]quinazoline (**37**) (0.32 g, 67%): mp (trihydrochloride salt from MeOH/Et_2O) 296 °C dec; ^{1}H NMR [trihydrochloride salt, $(CD_3)_2SO$] δ 11.37 (s, 1 H, NH), 11.17 (br s, 1 H, NH), 8.81 (s, 1 H, H-2), 8.25 (d, J = 2.0 Hz, 1 H, H-2′ or H-5), 8.02 (d, J = 8.4 Hz, 1 H, H-5′), 7.96 (dd, J = 8.4, 2.0 Hz, 1 H, H-6′), 7.87 (d, J = 9.0 Hz, 1 H, H-8), 7.78 (d, J = 2.1 Hz, 1 H, H-2′ or H-5), 7.53 (dd, J = 9.0, 2.2 Hz, 1 H, H-7), 5.46 (br s, 4 H, NH), 4.47 (d, J = 5.3 Hz, 2 H, $CH_2N^+H(CH_3)_2$), 2.79 (d, J = 4.6 Hz, 6 H, $N^+H(CH_3)_2$). Anal. ($C_{17}H_{18}BrN_5\cdot3HCl\cdot1.5H_2O$) C, H, N.

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) (2 mol equiv, 1.31 mmol, 250 mg) was added to a stirred solution of **37** (243 mg, 0.65 mmol), acrylic acid (4 mol equiv, 2.61 mmol, 179 μL), and pyridine (excess, 1.0 mL) in DMA (15 mL) under N_2. After 2 h at room temperature, the reaction mixture was concentrated under reduced pressure before being diluted with saturated $NaHCO_3$, the resulting suspension was then 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 EtOAc/CH_2Cl_2 (1:1) to MeOH/CH_2Cl_2/EtOAc (3:7:10), to give **5** (115 mg, 41%): mp (CH_2Cl_2) 206 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.52 (s, 1 H, CONH), 9.91 (s, 1 H, NH), 8.82 (d, J = 2.1 Hz, 1 H, H-5), 8.58 (s, 1 H, H-2), 8.20 (d, J = 2.1 Hz, 1 H, H-2′), 7.90 (dd, J = 8.9, 2.1 Hz, 1 H, H-7), 7.87 (dd, J = 8.4, 2.1 Hz, 1 H, H-6′), 7.80 (d, J = 8.9 Hz, 1 H, H-8), 7.44 (d, J = 8.4 Hz, 1 H, H-5′), 6.54 (dd, J = 17.0, 10.1 Hz, 1 H, CH=CH_2), 6.35 (dd, J = 17.0, 1.9 Hz, 1 H, CH=CH_2), 5.84 (dd, J = 10.1, 1.9 Hz, 1 H, CH=CH_2), 3.46 (s, 2 H, $CH_2N(CH_3)_2$), 2.22 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{20}H_{20}$-$BrN_5O\cdot H_2O$) C, H, N.

***N*-[4-[*N*-[3-Bromo-4-[2-(dimethylamino)ethoxy]phenyl]-amino]quinazolin-6-yl]acrylamide (6).** Reaction of 4-chloro-6-nitroquinazoline[17] (**34**) with 4-amino-2-bromo-1-[2-(dimethylamino)ethoxy]benzene (**33**) gave crude **36**, which was reduced with iron dust to crude **38**. EDCI·HCl-promoted acylation of this as described above gave **6**: mp (CH_2Cl_2/hexane) 181−184.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.50 (s, 1 H, CONH), 9.81 (s, 1 H, NH), 8.80 (br s, 1 H, H-5), 8.52 (s, 1 H, H-2), 8.09 (d, J = 2.6 Hz, 1 H, H-2′), 7.88 (dd, J = 1.9, 9.0 Hz, 1 H, H-7), 7.78 (partially obscured d, J = 9.0 Hz, 1 H, H-8), 7.76 (partially obscured dd, J = 9.0, 2.6 Hz, 1 H, H-6′), 7.17 (d, J = 9.0 Hz, 1 H, H-5′), 6.53 (dd, J = 16.9, 10.1 Hz, 1 H, CH=CH_2), 6.35 (dd, J = 16.9, 1.8 Hz, 1 H, CH=CH_2), 5.84 (dd, J = 10.1, 1.8 Hz, 1 H, CH=CH_2), 4.14 (t, J = 5.7 Hz, 2 H, OCH_2CH_2N), 2.69 (t, J = 5.7 Hz, 2 H, OCH_2CH_2N), 2.27 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{21}H_{22}BrN_5O_2\cdot0.25H_2O$) C, H, N.

***N*-[4-[*N*-(3-Bromophenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]quinazolin-6-yl]acrylamide (7). Example of the Method of Scheme 2**. Sodium metal (0.30 g, 13.2 mmol) was added to a solution of 3-(4-methyl-1-piperazinyl)-1-propanol (1.39 g, 8.81 mmol) in THF (40 mL) under N_2, and the resulting suspension was stirred at 20 °C for 2 h and then cannulated into a solution of 4-[(3-bromophenyl)amino]-7-fluoro-6-nitroquinazoline[29] (**39**) (0.80 g, 2.20 mmol) in THF (30 mL) under N_2. The reaction mixture was refluxed for 18 h before the solvent was partially removed under reduced pressure and the residue was diluted with water and extracted with EtOAc. The combined organic extracts were dried (Na_2-SO_4), concentrated under reduced pressure and chromatographed on silica gel, eluting with MeOH/CH_2Cl_2/EtOAc (1:9:10) to MeOH/CH_2Cl_2/EtOAc (2:3:5) to give 4-[*N*-(3-bromophenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]-6-nitroquinazoline (**43a**) (0.36 g, 33%): mp (trihydrochloride salt) (MeOH/Et_2O) 233 °C dec; ^{1}H NMR [free base, $(CD_3)_2SO$] δ 10.12 (s, 1 H, NH), 9.24 (s, 1 H, H-5), 8.69 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.88 (br d, J = 7.8 Hz, 1 H, H-6′), 7.47 (s, 1 H, H-8), 7.38 (t, J = 7.8 Hz, 1 H, H-5′), 7.34 (ddd, J = 7.8, 1.3, 1.3 Hz, 1 H, H-4′), 4.33 (t, J = 6.1 Hz, 2 H, $CH_2CH_2CH_2O$), 2.45 (t, J = 7.0 Hz, 2 H, $NCH_2CH_2CH_2$), 2.42−2.29 (br s, 8 H, piperazinyl methylene), 2.15 (s, 3 H, CH_3N), 1.92 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{22}H_{25}BrN_6O_3\cdot3HCl\cdot H_2O$) C, H, N, Cl.

Reduction of **43a** (0.31 g, 0.62 mmol) with Fe powder in EtOH/H_2O (2:1, 50 mL) containing glacial AcOH (1.0 mL) as above, followed by chromatography of the product on grade III alumina, eluting with MeOH/EtOAc (5:95), gave 6-amino-4-[*N*-(3-bromophenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]quinazoline (**47a**) (238 mg, 82%): mp (CH_2Cl_2) 171−172 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.36 (s, 1 H, NH), 8.38 (s, 1 H, H-2), 8.22 (t, J = 1.9 Hz, 1 H, H-2′), 7.86 (ddd, J = 8.2, 1.9, 0.8 Hz, 1 H, H-6′), 7.40 (s, 1 H, H-8), 7.30 (t, J = 8.0 Hz, 1 H, H-5′), 7.20 (ddd, J = 8.3, 1.9, 1.0 Hz, 1 H, H-4′), 7.09 (s, 1 H, H-5), 5.34 (s, 2 H, NH_2), 4.19 (t, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2O$), 2.49 (obscured t, $J \sim 7$ Hz, 2 H, $NCH_2CH_2CH_2$), 2.43−2.29 (br s, 8 H, piperazinyl methylene), 2.16 (s, 3 H, CH_3N), 1.97 (quintet, J = 6.8 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{22}H_{27}BrN_6O\cdot$ $1.25H_2O$) C, H, N.

A stirred solution of **47a** (223 mg, 0.47 mmol), acrylic acid (6 mol equiv, 2.84 mmol, 195 μL) and Et_3N (excess, 1.0 mL) in DMA (20 mL) under N_2 was treated with EDCI·HCl (3 mol

equiv, 1.42 mmol, 273 mg). Standard workup, followed by chromatography on grade III alumina, eluting with EtOAc/hexane (1:1) to MeOH/EtOAc (2:98), gave **7** (145 mg, 58%): mp (CH_2Cl_2/Et_2O/hexane) 105−107 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (s, 1 H, CONH), 9.61 (s, 1 H, NH), 8.89 (s, 1 H, H-5), 8.56 (s, 1 H, H-2), 8.17 (t, J = 1.9 Hz, 1 H, H-2′), 7.87 (br d, J = 8.3 Hz, 1 H, H-6′), 7.34 (t, J = 8.3 Hz, 1 H, H-5′), 7.28 (s, 1 H, H-8), 7.27 (obscured ddd, $J \sim 8$, ~ 1, ~ 1 Hz, 1 H, H-4′), 6.72 (dd, J = 17.0, 10.3 Hz, 1 H, C*H*═CH_2), 6.32 (dd, J = 17.0, 1.9 Hz, 1 H, CH═C*H*$_2$), 5.83 (dd, J = 10.3, 1.9 Hz, 1 H, CH═C*H*$_2$), 4.26 (t, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2O$), 2.47 (t, J = 7.1 Hz, 2 H, $NCH_2CH_2CH_2$), 2.42−2.27 (br s, 8 H, piperazinyl methylene), 2.15 (s, 3 H, CH_3N), 1.98 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{25}H_{29}BrN_6O_2$·0.5H_2O) C, H, N.

***N*-[4-[*N*-(3-Bromophenyl)amino]-7-[3-(4-morpholinyl)-propoxy]quinazolin-6-yl]acrylamide (8).** Sodium metal (0.63 g, 27.6 mmol) was added to a solution of 3-(4-morpholinyl)-1-propanol (3.20 g, 22.0 mmol) in THF (60 mL) under N_2. The resulting suspension was stirred at 20 °C for 2 h and then cannulated into a solution of **39**[29] (2.0 g, 5.51 mmol) in THF (50 mL) under N_2. Identical reaction procedure and workup as above gave, after chromatography on silica gel eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (2:3:5), 4-[*N*-(3-bromophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-nitroquinazoline (**43b**) (1.75 g, 65%): mp (MeOH) 216−220 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.12 (s, 1 H, NH), 9.24 (s, 1 H, H-5), 8.69 (s, 1 H, H-2), 8.19 (t, J = 1.8 Hz, 1 H, H-2′), 7.88 (ddd, J = 8.0, 1.4, 1.4 Hz, 1 H, H-6′), 7.49 (s, 1 H, H-8), 7.38 (t, J = 8.0 Hz, 1 H, H-5′), 7.34 (ddd, J = 8.0, 1.4, 1.4 Hz, 1 H, H-4′), 4.35 (t, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2O$), 3.58 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.45 (t, J = 7.0 Hz, 2 H, NCH_2CH_2-CH_2), 2.37 (m, 4 H, morpholino methylene), 1.94 (quintet, J = 6.6 Hz, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR δ 157.76, 157.26, 153.76, 153.21, 140.32, 138.86, 130.37, 126.38, 124.26, 121.70, 121.13, 120.72, 110.11, 107.88, 67.87, 66.13 (×2), 54.42, 53.28 (×2), 25.30. Anal. ($C_{21}H_{22}BrN_5O_4$·0.75H_2O) C, H, N.

Reduction of **43b** (1.50 g, 3.07 mmol) with Fe powder in EtOH/H_2O (2:1, 80 mL) containing glacial AcOH (2.0 mL) as above, followed by chromatography of the product on grade III alumina eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/EtOAc (2:98), gave 6-amino-4-[*N*-(3-bromophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazoline (**47b**) (1.08 g, 77%): mp (EtOAc/hexane) 158−160 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.37 (s, 1 H, NH), 8.40 (s, 1 H, H-2), 8.24 (t, J = 1.9 Hz, 1 H, H-2′), 7.86 (ddd, J = 8.2, 1.9, 0.9 Hz, 1 H, H-6′), 7.42 (s, 1 H, H-8), 7.30 (t, J = 8.2 Hz, 1 H, H-5′), 7.21 (ddd, J = 8.2, 1.9, 0.9 Hz, 1 H, H-4′), 7.09 (s, 1 H, H-5), 5.36 (s, 2 H, NH_2), 4.20 (t, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2O$), 3.59 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.50 (t, J = 7.3 Hz, 2 H, $NCH_2CH_2CH_2$), 2.39 (m, 4 H, morpholino methylene), 1.99 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR δ 154.88, 151.94, 150.19, 144.84, 141.94, 138.50, 130.16, 124.66, 123.02, 121.09, 119.65, 110.42, 106.37, 100.81, 66.45, 66.14 (×2), 54.77, 53.29 (×2), 25.50. Anal. ($C_{21}H_{24}BrN_5O_2$·0.25H_2O) C, H, N.

A stirred solution of **47b** (0.50 g, 1.09 mmol), acrylic acid (6 mol equiv, 6.54 mmol, 449 μL), and Et_3N (excess, 2.0 mL) in DMF (20 mL) under N_2 was treated with EDCI·HCl (3 mol equiv, 3.27 mmol, 627 mg). The standard procedure above was followed to give, after chromatography on grade III alumina eluting with EtOAc/hexane (9:1) to MeOH/EtOAc (2:98), **8** (329 mg, 59%): mp (EtOAc/Et_2O/hexane) 170−172 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (s, 1 H, CONH), 9.62 (s, 1 H, NH), 8.89 (s, 1 H, H-5), 8.56 (s, 1 H, H-2), 8.18 (t, J = 1.9 Hz, 1 H, H-2′), 7.88 (br d, J = 8.2 Hz, 1 H, H-6′), 7.34 (t, J = 8.1 Hz, 1 H, H-5′), 7.30 (s, 1 H, H-8), 7.27 (ddd, J = 7.9, 1.4, 0.8 Hz, 1 H, H-4′), 6.72 (dd, J = 17.0, 10.2 Hz, 1 H, C*H*═CH_2), 6.33 (dd, J = 17.0, 1.9 Hz, 1 H, CH═C*H*$_2$), 5.83 (dd, J = 10.2, 1.9 Hz, 1 H, CH═C*H*$_2$), 4.27 (t, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2O$), 3.58 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.48 (t, J = 7.1 Hz, 2 H, $NCH_2CH_2CH_2$), 2.38 (m, 4 H, morpholino methylene), 1.99 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR δ 163.49, 156.68, 154.96, 153.92, 149.19, 141.20, 131.58, 130.19, 127.16, 126.95, 125.52, 123.97, 121.03, 120.52, 116.78, 108.80, 107.28, 66.96, 66.14 (×2), 54.54, 53.28 (×2), 25.31. Anal. ($C_{24}H_{26}$-BrN_5O_3·0.5H_2O) C, H, N.

***N*-[4-[*N*-(3-Bromophenyl)amino]-7-[4-(*N*,*N*-dimethylamino)butoxy]quinazolin-6-yl]acrylamide (9).** To a suspension of hexane-washed sodium hydride (11.0 mmol, 440 mg of a 60% dispersion in mineral oil) in THF (20 mL) was cannulated a solution of 4-(*N*,*N*-dimethylamino)-1-butanol (8.80 mmol, 1.03 g) in THF (30 mL). The resulting suspension was stirred at 20 °C under N_2 for 2 h and then cannulated into a solution of **39** (0.80 g, 2.20 mmol) in THF (30 mL) under N_2. The dark red solution was heated at reflux overnight, then worked up as above. Chromatography of the crude product on grade III alumina eluting with EtOAc to MeOH/EtOAc (5:95), gave 6-amino-4-[*N*-(3-bromophenyl)amino]-7-[4-(*N*,*N*-dimethylamino)butoxy]quinazoline (**47c**) (310 mg, 33%): mp (CH_2Cl_2/hexane) 155−156 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.36 (s, 1 H, NH), 8.39 (s, 1 H, H-2), 8.23 (t, J = 1.9 Hz, 1 H, H-2′), 7.86 (br d, J = 8.0 Hz, 1 H, H-6′), 7.41 (s, 1 H, H-8), 7.30 (t, J = 8.1 Hz, 1 H, H-5′), 7.20 (ddd, J = 8.2, 1.9, 0.8 Hz, 1 H, H-4′), 7.09 (s, 1 H, H-5), 5.32 (s, 2 H, NH_2), 4.17 (t, J = 6.2 Hz, 2 H, CH_2CH_2-CH_2CH_2O), 2.47 (t, J = 7.3 Hz, 2 H, $NCH_2CH_2CH_2CH_2$), 2.15 (s, 6 H, $N(CH_3)_2$), 1.84 (quintet, J = 6.4 Hz, 2 H, $CH_2CH_2CH_2$-CH_2), 1.62 (quintet, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2CH_2$). Anal. ($C_{20}H_{24}BrN_5O$·0.5H_2O) C, H, N.

A solution of **47c** (276 mg, 0.64 mmol), acrylic acid (6 mol equiv, 3.85 mmol, 264 μL), and Et_3N (excess, 1.0 mL) in DMA (10 mL) under N_2 was treated with EDCI·HCl (3 mol equiv, 1.92 mmol, 369 mg). The standard procedure above, followed by chromatography of the product on grade III alumina eluting with EtOAc/hexane (1:1) to MeOH/EtOAc (3:97), gave **9** (98 mg, 32%): mp (CH_2Cl_2/Et_2O) 112−115 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 9.77 (s, 1 H, CONH), 9.62 (s, 1 H, NH), 8.88 (s, 1 H, H-5), 8.56 (s, 1 H, H-2), 8.17 (t, J = 1.9 Hz, 1 H, H-2′), 7.87 (ddd, J = 8.2, 1.9, 1.0 Hz, 1 H, H-6′), 7.34 (t, J = 8.2 Hz, 1 H, H-5′), 7.29 (s, 1 H, H-8), 7.27 (ddd, J = 8.2, 1.9, 1.0 Hz, 1 H, H-4′), 6.71 (dd, J = 17.1, 10.2 Hz, 1 H, C*H*═CH_2), 6.32 (dd, J = 17.1, 1.9 Hz, 1 H, CH═C*H*$_2$), 5.82 (dd, J = 10.2, 1.9 Hz, 1 H, CH═C*H*$_2$), 4.24 (t, J = 6.6 Hz, 2 H, $CH_2CH_2CH_2CH_2O$), 2.27 (t, J = 7.2 Hz, 2 H, $NCH_2CH_2CH_2CH_2$), 2.12 (s, 6 H, $N(CH_3)_2$), 1.85 (quintet, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2CH_2$), 1.60 (quintet, J = 7.4 Hz, 2 H, $CH_2CH_2CH_2CH_2$). Anal. ($C_{23}H_{26}BrN_5O_2$·1.25H_2O) C, H, N.

***N*-[4-[*N*-(3-Bromophenyl)amino]-7-[3-(1*H*-imidazol-1-yl)propoxy]quinazolin-6-yl]acrylamide (10).** To a suspension of hexane-washed sodium hydride (5.50 mmol, 220 mg of a 60% dispersion in mineral oil) in THF (20 mL) was cannulated a solution of 3-(1*H*-imidazol-1-yl)-1-propanol (4.84 mmol, 0.61 g) in THF (30 mL). The resulting suspension was stirred at 20 °C under N_2 for 2 h during which time the required sodium alkoxide partially precipitated from solution. Solid **39** (0.80 g, 2.20 mmol) was then added, and the resulting dark red solution was heated at reflux for 24 h, then worked up as above. Chromatography of the product on silica gel, eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (3:7:10), gave 4-[*N*-(3-bromophenyl)amino]-7-[3-(1*H*-imidazol-1-yl)propoxy]-6-nitroquinazoline (**43d**) (524 mg, 51%): mp (CH_2Cl_2/hexane) 212−215 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.16 (s, 1 H, NH), 9.30 (s, 1 H, H-5), 8.70 (s, 1 H, H-2), 8.19 (t, J = 1.6 Hz, 1 H, H-2′), 7.88 (ddd, J = 7.8, 1.5, 1.6 Hz, 1 H, H-6′), 7.63 (s, 1 H, imidazolyl methine), 7.48 (s, 1 H, H-8), 7.39 (t, J = 7.9 Hz, 1 H, H-5′), 7.35 (ddd, J = 8.0, 1.6, 1.6 Hz, 1 H, H-4′), 7.21 (s, 1 H, imidazolyl methine), 6.90 (s, 1 H, imidazolyl methine), 4.22 (t, J = 6.0 Hz, 2 H, $CH_2CH_2CH_2$), 4.18 (t, J = 6.8 Hz, 2 H, $CH_2CH_2CH_2$), 2.26 (quintet, J = 6.4 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{20}H_{17}BrN_6O_3$) C, H, N.

Reduction of **43d** (0.51 g, 1.08 mmol) with Fe (0.24 g, 4 mol equiv) in refluxing EtOH/H_2O (2:1, 60 mL) containing glacial AcOH (0.7 mL) as above, followed by chromatography on grade III alumina eluting with MeOH/EtOAc (5:95), gave 6-amino-4-[*N*-(3-bromophenyl)amino]-7-[3-(1*H*-imidazol-1-yl)propoxy]-quinazoline (**47d**) (389 mg, 82%): mp (CH_2Cl_2/Et_2O) 178−180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.37 (s, 1 H, NH), 8.38 (s, 1 H, H-2), 8.22 (t, J = 1.8 Hz, 1 H, H-2′), 7.86 (br d, J = 8.1 Hz, 1 H, H-6′), 7.66 (s, 1 H, imidazolyl methine), 7.40 (s, 1 H, H-8), 7.30

(t, J = 8.1 Hz, 1 H, H-5′), 7.23 (s, 1 H, imidazolyl methine), 7.21 (br d, J = 7.7 Hz, 1 H, H-4′), 7.06 (s, 1 H, H-5), 6.90 (s, 1 H, imidazolyl methine), 5.45 (s, 2 H, NH_2), 4.28 (t, J = 7.1 Hz, 2 H, $CH_2CH_2CH_2$), 4.10 (t, J = 5.8 Hz, 2 H, $CH_2CH_2CH_2$), 2.27 (quintet, J = 6.5 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{20}H_{19}BrN_6O\cdot 0.5H_2O$) C, H, N.

A stirred solution of **47d** (383 mg, 0.87 mmol), acrylic acid (6 mol equiv, 5.23 mmol, 359 μL), and pyridine (excess, 1.0 mL) in DMA (20 mL) under N_2 was treated with EDCI·HCl (5 mol equiv, 4.36 mmol, 838 mg). The standard procedure above, followed by chromatography on grade III alumina eluting with EtOAc/hexane (1:1) to MeOH/EtOAc (5:95), gave **10** (9 mg, 2%): mp (CH_2Cl_2/Et_2O/hexane) 235−237 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.79 (s, 1 H, CONH), 9.60 (s, 1 H, NH), 8.88 (s, 1 H, H-5), 8.55 (s, 1 H, H-2), 8.18 (t, J = 1.9 Hz, 1 H, H-2′), 7.87 (ddd, J = 8.1, 1.9, 1.0 Hz, 1 H, H-6′), 7.64 (s, 1 H, imidazolyl methine), 7.34 (t, J = 8.1 Hz, 1 H, H-5′), 7.28 (partially obscured ddd, $J \sim$ 8, 1.2, 1.2 Hz, 1 H, H-4′), 7.27 (s, 1 H, H-5), 7.21 (t, J = 1.3 Hz, 1 H, imidazolyl methine), 6.89 (br s, 1 H, imidazolyl methine), 6.73 (dd, J = 17.0, 10.2 Hz, 1 H, $CH{=}CH_2$), 6.34 (dd, J = 17.0, 1.8 Hz, 1 H, $CH{=}CH_2$), 5.85 (dd, J = 10.2, 1.8 Hz, 1 H, $CH{=}CH_2$), 4.22 (t, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2$), 4.14 (t, J = 6.0 Hz, 2 H, $CH_2CH_2CH_2$), 2.27 (quintet, J = 6.4 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{23}H_{21}BrN_6O_2\cdot 0.75H_2O$) C, H, N.

***N*-[4-[*N*-(3-Bromophenyl)amino]-7-[3-(*N*,*N*-diethylamino)propylthio]quinazolin-6-yl]acrylamide (11).** 3-(*N*,*N*-Diethylamino)propanethiol hydrochloride (422 mg, 2.20 mmol) was added to a slurry of hexane-washed NaH (60% oil suspension, 320 mg, 8.00 mmol) in DMSO (2 mL) stirred under N_2 at 25 °C. After 20 min, a solution of **39** (726 mg, 2.0 mmol) in DMSO (3 mL) was added, and the dark mixture was heated to 65 °C for 30 min, then poured onto ice-cold saturated $NaHCO_3$ solution. The precipitate was collected by filtration, rinsed with water, and dried to give 4-[*N*-(3-bromophenyl)-amino]-7-[3-(*N*,*N*-diethylamino)propylthio]-6-nitroquinazoline (**43e**) (940 mg, 100%) as a brown solid, that was used directly; ^{1}H NMR [$(CD_3)_2SO$] δ 9.28 (s, 1 H, H-5), 8.34 (s, 1 H, H-2), 7.87 (s, 1 H, H-2′), 7.52 (d, J = 7.6 Hz, H-6′), 7.41 (s, 1 H, H-8), 7.21 (t, 1 H, J = 8.0 Hz, H-5′), 7.12 (d, 1 H, J = 8.1 Hz, H-4′), 3.05 (t, 1 H, J = 7.2 Hz, SCH_2), 2.47 (t, 2 H, J = 7 Hz, NCH_2), 2.40 (q, 4 H, J = 7.1 Hz, NCH_2), 1.72 (quintet, 2 H, J = 7.1 Hz, CH_2), 0.90 (t, 6 H, J = 7.2 Hz, Me).

Iron powder (446 mg, 8.0 mmol) was added to a vigorously stirred mixture of **43e** (940 mg, 2.00 mmol) in refluxing EtOH/water/AcOH (8:4:0.5 mL). After 30 min the reaction mixture was allowed to cool to room temperature, and was then neutralized with concentrated aqueous ammonia, filtered through a pad of Celite, and the residue rinsed with EtOH. The combined filtrates were concentrated to small volume and extracted with EtOAc. The volatiles were removed rigorously under reduced pressure to give 6-amino-4-[*N*-(3-bromophenyl)-amino]-7-[3-(*N*,*N*-diethylamino)propylthio]quinazoline (**47e**) (604 mg, 66%) as a brown solid, that was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 9.48 (s, 1 H, NH), 8.34 (s, 1 H, H-2), 8.16 (s, 1 H, H-2′), 7.81 (d, J = 7.1 Hz, H-6′), 7.51 (s, 1 H, H-5), 7.41 (s, 1 H, H-8), 7.26 (t, 1 H, J = 8.0 Hz, H-5′), 7.18 (d, 1 H, J = 7.8 Hz, H-4′), 5.42 (br s, 2 H, NH_2), 3.03 (t, 1 H, J = 6.8 Hz, SCH_2), 2.44 (t, 2 H, J = 7 Hz, NCH_2), 2.39 (q, 4 H, J = 7.1 Hz, NCH_2), 1.68 (quintet, 2 H, J = 6.6 Hz, CH_2), 0.89 (t, 6 H, J = 6.8 Hz, CH_3).

A stirred solution of **47e** (604 mg, 1.30 mmol) and acrylic acid (0.19 mL, 2.8 mmol) and pyridine (0.22 mL, 2.7 mmol) in DMF (5 mL) was treated with EDCI·HCl (540 mg, 2.8 mmol) under N_2 at 0 °C, and reaction mixture was then stirred at 25 °C for 3 h before being quenched by the addition of water. The mixture was extracted with EtOAc, and the combined extracts were washed with water, saturated brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was flash chromatographed on silica gel, eluting with 2−20% MeOH in CH_2Cl_2, and the appropriate fractions were triturated with Me_2CO to give **11** (116 mg, 17%) as a light brown glass: mp 77−79 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.88 (s, 1 H, NH), 9.78 (s, 1 H, NH), 8.57 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.16 (t, 1 H, J = 1.9 Hz, H-2′), 7.85 (d, J = 8.8 Hz, H-6′), 7.56 (s, 1 H, H-8), 7.27 (t, 1 H, J = 7.9 Hz, H-5′), 7.21 (d, 1 H, J = 8.3 Hz, H-4′), 6.54 (dd, 1 H, J = 17.5, 10.5 Hz, $CH{=}CH_2$), 6.25 (dd, 1 H, J = 1.7, 17.0 Hz, $CH{=}CH_2$), 5.77 (d, 1 H, J = 10.3 Hz, $CH{=}CH_2$), 3.07 (t, 1 H, J = 7.2 Hz, SCH_2), 2.47 (t, 2 H, J = 6.8 Hz, NCH_2), 2.41 (q, 4 H, J = 7.1 Hz, NCH_2), 1.72 (quintet, 2 H, J = 6.9 Hz, CH_2), 0.91(t, 6 H, J = 7.1 Hz, CH_3); MS (APCI) 516 (95, $^{81}BrMH^+$), 514 (100, $^{79}BrMH^+$). Anal. ($C_{24}H_{28}N_5OBr\cdot H_2O\cdot 0.5CH_2Cl_2$) C, H, N.

***N*-[4-[*N*-(3-Methylphenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]quinazolin-6-yl]acrylamide (13).** A solution of 4-chloro-7-fluoro-6-nitroquinazoline (prepared as described[29] from 7-fluoro-6-nitroquinazolone) was dissolved in dry CH_2Cl_2 (50 mL) and added to a stirred solution of 3-methylaniline in *i*-PrOH (30 mL). The reaction mixture was stirred at 20 °C for 30 min and then diluted with hexane (200 mL). The resulting precipitate was collected, washed with hexane and dissolved in MeOH/H_2O (4:1, 150 mL) with gentle warming. The solution was treated with excess Et_3N and diluted with water (400 mL), and the precipitate was filtered, washed with water and dried under reduced pressure to give 7-fluoro-4-[*N*-(3-methylphenyl)amino]-6-nitroquinazoline (**40**) (3.01 g, 88%): mp (CH_2Cl_2/hexane) 191−192 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.38 (s, 1 H, NH), 9.62 (d, J_{H-F} = 8.1 Hz, 1 H, H-5), 8.67 (s, 1 H, H-2), 7.80 (d, J_{H-F} = 12.6 Hz, 1 H, H-8), 7.63 (br d, J = 8.2 Hz, 1 H, H-6′), 7.60 (br s, 1 H, H-2′), 7.31 (t, J = 7.8 Hz, 1 H, H-5′), 7.03 (br d, J = 7.5 Hz, 1 H, H-4′), 2.35 (s, 3 H, $ArCH_3$). Anal. ($C_{15}H_{11}FN_4O_2$) C, H, N.

Sodium metal (0.23 g, 10.1 mmol) was added to a solution of 3-(4-methyl-1-piperazinyl)-1-propanol (1.06 g, 6.71 mmol) in THF (15 mL) under N_2. The resulting suspension was stirred at 20 °C for 2 h and then cannulated into a solution of **40** (0.50 g, 1.68 mmol) in THF (20 mL) under N_2. Standard workup followed by chromatography of the product on alumina, eluting with EtOAc/hexane (1:1) to EtOAc (2:3:5), gave 4-[*N*-(3-methylphenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]-6-nitroquinazoline (**44a**) (0.67 g, 91%): mp (Et_2O/hexane) 155−156 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.00 (s, 1 H, NH), 9.26 (s, 1 H, H-5), 8.61 (s, 1 H, H-2), 7.64 (br d, J = 8.4 Hz, 1 H, H-6′), 7.62 (br s, 1 H, H-2′), 7.43 (s, 1 H, H-8), 7.29 (t, J = 7.8 Hz, 1 H, H-5′), 6.99 (br d, J = 7.4 Hz, 1 H, H-4′), 4.32 (t, J = 6.0 Hz, 2 H, $CH_2CH_2CH_2O$), 2.44 (t, J = 7.0 Hz, 2 H, $NCH_2CH_2CH_2$), 2.39−2.28 (br s, 8 H, piperazinyl methylene), 2.34 (s, 3 H, CH_3-Ar), 2.14 (s, 3 H, CH_3N), 1.92 (quintet, J = 6.6 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{23}H_{28}N_6O_3$) C, H, N.

A solution of **44a** (0.61 g, 1.40 mmol) in MeOH/EtOAc (2:1, 50 mL) was hydrogenated (60 psi) over Pd−C for 5 h and then filtered through Celite. The filtrate was concentrated under reduced pressure and chromatographed on grade III alumina, eluting with MeOH/EtOAc (5:95) to give 6-amino-4-[*N*-(3-methylphenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]-quinazoline (**48a**) (361 mg), which appeared unstable (rapid discoloration) and was used without further characterization. A stirred solution of **48a** (0.36 g, 0.89 mmol), acrylic acid (6 mol equiv, 5.53 mmol, 366 μL), and Et_3N (excess, 2.0 mL) in DMF (20 mL) under N_2 was treated with EDCI·HCl (3 mol equiv, 2.66 mmol, 511 mg). Standard workup followed by chromatography on grade III alumina, eluting with EtOAc to MeOH/EtOAc (2:98), gave **13** (65 mg, 16%): mp (Et_2O/hexane) 60−66 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.60 (s, 1 H, exchangeable), 9.59 (s, 1 H, exchangeable), 8.86 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 7.62 (partially obscured br d, J = 8.0 Hz, 1 H, H-6′), 7.62 (br s, 1 H, H-2′), 7.25 (partially obscured t, J = 8.1 Hz, 1 H, H-5′), 7.25 (s, 1 H, H-8), 6.92 (br d, J = 7.5 Hz, 1 H, H-4′), 6.70 (dd, J = 17.0, 10.2 Hz, 1 H, $CH{=}CH_2$), 6.31 (dd, J = 17.0, 1.8 Hz, 1 H, $CH{=}CH_2$), 5.83 (dd, J = 10.2, 1.8 Hz, 1 H, $CH{=}CH_2$), 4.24 (t, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2O$), 2.47 (t, J = 7.1 Hz, 2 H, $NCH_2CH_2CH_2$), 2.41−2.28 (br s, 8 H, piperazinyl methylene), 2.33 (s, 3 H, CH_3Ar), 2.15 (s, 3 H, CH_3N), 1.97 (quintet, J = 6.8 Hz, 2 H, $CH_2CH_2CH_2$). EI HRMS (M^+) $C_{26}H_{32}N_6O_2$: Requires 460.2587. Found: 460.2576.

***N*-[4-[*N*-(3-Methylphenyl)amino]-7-[3-(4-morpholinyl)-propoxy]quinazolin-6-yl]acrylamide (14).** Sodium metal (0.27 g, 11.8 mmol) was added to a solution of 3-(4-morpholi-

nyl)-1-propanol (1.22 g, 8.40 mmol) in THF (40 mL) under N_2. The resulting suspension was stirred at 20 °C for 2 h and then cannulated into a solution of **40** (0.70 g, 2.35 mmol) in THF (30 mL) under N_2. Reaction and workup as above, followed by chromatography on silica gel, eluting with MeOH/CH_2Cl_2/EtOAc (5:45:50) to MeOH/CH_2Cl_2/EtOAc (3:7:10), gave 4-[*N*-(3-methylphenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-nitroquinazoline (**44b**) (0.87 g, 88%): mp (CH_2Cl_2/hexane) 169−170 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.00 (s, 1 H, NH), 9.26 (s, 1 H, H-5), 8.62 (s, 1 H, H-2), 7.64 (br d, J = 8.1 Hz, 1 H, H-6′), 7.62 (br s, 1 H, H-2′), 7.45 (s, 1 H, H-8), 7.29 (t, J = 7.8 Hz, 1 H, H-5′), 6.99 (br d, J = 7.5 Hz, 1 H, H-4′), 4.34 (t, J = 6.1 Hz, 2 H, $CH_2CH_2CH_2O$), 3.58 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.46 (t, J = 7.0 Hz, 2 H, $NCH_2CH_2CH_2$), 2.38 (br s, 4 H, morpholino methylene), 2.35 (s, 3 H, CH_3Ar), 1.94 (quintet, J = 6.6 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{22}H_{25}N_5O_4$) C, H, N.

A solution of **44b** (0.71 g, 1.68 mmol) in MeOH/EtOAc (2:1, 60 mL) was hydrogenated (60 psi) over Pd−C for 6 h and then filtered through Celite and worked up as above to to give 6-amino-4-[*N*-(3-methylphenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazoline (**48b**), which was used without further characterization. A stirred solution of **48b** (0.7 g, 1.8 mmol), acrylic acid (6 mol equiv, 10.8 mmol, 776 μL), and Et_3N (excess, 4.0 mL) in DMF (20 mL) under N_2 was treated with EDCI·HCl (3 mol equiv, 5.38 mmol, 1.03 g). Standard workup and chromatography on silica gel, eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (3:7:10), gave **14** (175 mg, 22%): mp (EtOAc/Et_2O) 69−72 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.60 (s, 1 H, exchangeable), 9.59 (s, 1 H, exchangeable), 8.86 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 7.62 (partially obscured br d, J = 8.0 Hz, 1 H, H-6′), 7.61 (br s, 1 H, H-2′), 7.26 (s, 1 H, H-8), 7.25 (t, J = 7.8 Hz, 1 H, H-5′), 6.92 (br d, J = 7.4 Hz, 1 H, H-4′), 6.70 (dd, J = 16.9, 10.2 Hz, 1 H, C*H*=CH_2), 6.32 (dd, J = 16.9, 1.9 Hz, 1 H, CH=CH_2), 5.82 (dd, J = 10.2, 1.9 Hz, 1 H, CH=CH_2), 4.26 (t, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2O$), 3.58 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.48 (t, J = 7.1 Hz, 2 H, $NCH_2CH_2CH_2$), 2.38 (m, 4 H, morpholino methylene), 2.33 (s, 3H, CH_3Ar), 1.99 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{25}H_{29}N_5O_3$·0.25H_2O) C, H, N.

***N*-[4-[*N*-(3-Bromo-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazolin-6-yl]acrylamide (16).** Reaction of 4-chloro-7-fluoro-6-nitroquinazoline[29] with 3-bromo-4-fluoroaniline, using the procedure previously described for 3-bromoaniline,[29] gave 4-[*N*-(3-bromo-4-fluorophenyl)amino]-7-fluoro-6-nitroquinazoline (**41**) (89%): mp (*i*-PrOH) 238−240 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.51 (s, 1 H, NH), 9.59 (d, J_{H-F} = 8.0 Hz, 1 H, H-5), 8.74 (s, 1 H, H-2), 8.24 (dd, J_{H-F} = 6.0 Hz, J = 2.2 Hz, 1 H, H-2'), 7.87 (m, 1H, H-6'), 7.86 (d, J_{H-F} = 12.5 Hz, 1 H, H-8), 7.46 (dd, J_{H-F} = 8.8 Hz, J = 8.8 Hz, H-5'). Anal. ($C_{14}H_7BrF_2N_4O_2$) C, H, N.

Treatment of **41** with the sodium salt of 3-(4-morpholinyl)-1-propanol, as described above, gave 4-[*N*-(3-bromo-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-nitroquinazoline (**45b**) (39%): mp (MeOH) 186−188 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.14 (s, 1 H, NH), 9.21 (s, 1 H, H-5), 8.67 (d, J = 1.8 Hz, 1 H, H-2), 8.26 (td, J_{H-F} = 6.0 Hz, J = 1.8, 1.8 Hz, 1 H, H-2′), 7.89−7.83 (m, 1H, H-6′), 7.48 (s, 1 H, H-8), 7.44 (t, J = 8.8 Hz, 1 H, H-5′), 4.35 (t, J = 6.1 Hz, 2 H, $CH_2CH_2CH_2O$), 3.58 (t, J = 4.5 Hz, 4 H, morpholino methylene), 2.45 (t, J = 6.6 Hz, 2 H, $CH_2CH_2CH_2N$), 2.38 (m, 4 H, morpholino methylene), 1.95 (quintet, J = 6.6 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{21}H_{21}BrFN_5O_4$) C, H, N.

Iron dust reduction of **45b** gave 6-amino-4-[*N*-(3-bromo-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazoline (**49b**) (91%): mp (*i*-Pr_2O/CH_2Cl_2) 149−150 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.37 (s, 1 H, NH), 8.37 (s, 1 H, H-2), 8.29 (dd, J_{H-F} = 6.3 Hz, J = 2.5 Hz, 1 H, H-2′), 7.88−7.84 (m, 1 H, H-6′), 7.38 (s, 1 H, H-8), 7.36 (dd, J_{H-F} = 8.8 Hz, J = 8.8 Hz, 1 H, H-5′), 7.08 (s, 1 H, H-5), 5.34 (s, 2 H, NH_2), 4.20 (t, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2O$), 3.59 (t, J = 4.5 Hz, 4 H, morpholino methylene), 2.50 (t, J = 7.3 Hz, 2 H, $CH_2CH_2CH_2N$), 2.40 (m, 4 H, morpholino methylene), 1.99 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{21}H_{23}BrFN_5O_2$) C, H, N.

Treatment of **49b** with acrylic acid and EDCI·HCl in DMA, as described above, gave **16** (49%): mp (MeOH) 171−173 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.80 (s, 1 H, CONH), 9.62 (s, 1 H, NH), 8.87 (s, 1 H, H-5), 8.54 (s, 1 H, H-2), 8.23 (dd, J_{H-F} = 5.1 Hz, J = 2.6 Hz, 1 H, H-2′), 7.88−7.84 (m, 1 H, H-6′), 7.40 (dd, J_{H-F} = 8.8 Hz, J = 8.8 Hz, 1 H, H-5′), 7.29 (s, 1 H, H-8), 6.72 (dd, J = 17.0, 10.2 Hz, 1 H, C*H*=CH_2), 6.32 (dd, J = 17.0, 1.9 Hz, 1 H, CH=CH_2), 5.83 (dd, J = 10.2, 1.9 Hz, 1 H, CH=CH_2), 4.27 (t, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2O$), 3.59 (t, J = 4.3 Hz, 4 H, morpholino methylene), 2.49 (t, J = 7.3 Hz, 2 H, $CH_2CH_2CH_2N$), 2.39 (m, 4 H, morpholino methylene), 2.00 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{24}H_{25}BrFN_5O_3$·H_2O) C, H, N.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazolin-6-yl]acrylamide (18).** Reaction of 4-chloro-7-fluoro-6-nitroquinazoline[29] with 3-chloro-4-fluoroaniline, as above, gave 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-fluoro-6-nitroquinazoline (**42**) (72%): mp (*i*-PrOH) 239−240 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.43 (s, 1 H, NH), 9.53 (d, J_{H-F} = 8.0 Hz, 1 H, H-5), 8.70 (s, 1 H, H-2), 8.11 (dd, J_{H-F} = 6.7 Hz, J = 2.4 Hz, 1 H, H-2'), 7.80 (d, J_{H-F} = 12.4 Hz, 1 H, H-8), 7.78 (m, 1H, H-6'), 7.46 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, H-5'). Anal. ($C_{14}H_7ClF_2N_4O_2$) C, H, N.

Treatment of **42** with the sodium salt of 3-(4-morpholinyl)-1-propanol, as described above, gave 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-nitroquinazoline (**46b**) (58%): mp (MeOH) 194−195 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.16 (s, 1 H, NH), 9.22 (s, 1 H, H-5), 8.68 (d, J = 1.8 Hz, 1 H, H-2), 8.17 (dd, J_{H-F} = 6.8 Hz, J = 2.5 Hz, 1 H, H-2′), 7.82−7.78 (m, 1H, H-6′), 7.49 (s, 1 H, H-8), 7.47 (dd, J_{H-F} = 8.9 Hz, J = 8.9 Hz, 1 H, H-5′), 4.35 (t, J = 6.1 Hz, 2 H, $CH_2CH_2CH_2O$), 3.57 (t, J = 4.5 Hz, 4 H, morpholino methylene), 2.46 (t, J = 7.0 Hz, 2 H, $CH_2CH_2CH_2N$), 2.37 (m, 4 H, morpholino methylene), 1.94 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{21}H_{21}ClFN_5O_4$) C, H, N.

Iron dust reduction of **46b** gave 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]quinazoline (**50b**) (78%): mp (*i*-Pr_2O/CH_2Cl_2) 166−167 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.38 (s, 1 H, NH), 8.37 (s, 1 H, H-2), 8.19 (dd, J_{H-F} = 6.9 Hz, J = 2.7 Hz, 1 H, H-2′), 7.83−7.79 (m, 1 H, H-6′), 7.39 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 7.38 (s, 1 H, H-8), 7.08 (s, 1 H, H-5), 5.36 (s, 2 H, NH_2), 4.20 (t, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2O$), 3.59 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.51 (t, J = 7.4 Hz, 2 H, $CH_2CH_2CH_2N$), 2.40 (m, 4 H, morpholino methylene), 1.99 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{21}H_{23}ClFN_5O_2$) C, H, N.

Treatment of **50b** with acrylic acid and EDCI·HCl in DMA, as described above, gave **18** (38%): mp (MeOH) 188−190 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.80 (s, 1 H, CONH), 9.62 (s, 1 H, NH), 8.87 (s, 1 H, H-5), 8.54 (s, 1 H, H-2), 8.14 (dd, J_{H-F} = 6.8 Hz, J = 2.6 Hz, 1 H, H-2′), 7.83−7.79 (m, 1 H, H-6′), 7.43 (dd, J_{H-F} = 9.2 Hz, J = 9.2 Hz, 1 H, H-5′), 7.30 (s, 1 H, H-8), 6.72 (dd, J = 17.1, 10.2 Hz, 1 H, C*H*=CH_2), 6.32 (dd, J = 17.1, 1.9 Hz, 1 H, CH=CH_2), 5.83 (dd, J = 10.2, 1.9 Hz, 1 H, CH=CH_2), 4.27 (t, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2O$), 3.58 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.48 (t, J = 7.1 Hz, 2 H, $CH_2CH_2CH_2N$), 2.38 (m, 4 H, morpholino methylene), 2.00 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{24}H_{25}ClFN_5O_3$·H_2O) C, H, N.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-(3,6,9-trioxadecanoxy)quinazolin-6-yl]acrylamide (19).** A mixture of **42** (673 mg, 2.00 mmol), triethylene glycol monomethyl ether (493 mg, 3.00 mmol) and potassium trimethylsilanolate (770 mg, 6.0 mmol) in DMSO (25 mL) was stirred together under N_2 for 5 h on a 25 °C water bath. The deep crimson mixture was poured onto stirred ice−water (40 mL), and the reddish precipitate was collected by Buchner filtration, rinsed with dilute Na_2CO_3 solution and water (2 × 10 mL), and was dried in a vacuum oven at 60 °C over P_2O_5 to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-nitro-7-(3,6,9-trioxadecanoxy)quinazoline (**46f**) (696 mg, 72%), which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 10.16 (br s, 1 H, NH), 9.19 (s, 1 H, H-5), 8.66 (s, 1 H, H-2), 8.15 (dd, 1 H, J = 2.3, 6.8 Hz, H-2′), 7.81−7.78 (m, 1 H, H-6′), 7.50 (s, 1 H, H-8), 7.46 (t, 1 H, J = 9.1 Hz, H-5′),

4.43 (t, 2 H, J = 4.2 Hz, $ArOCH_2$), 3.82 (t, 2 H, J = 4.2 Hz, $ArOCH_2CH_2$), 3.62, 3.53, 3.51, 3.41 (dds, 2 H, 2 H, 2 H, 2 H, $J \sim 4$, 6 Hz, OCH_2s), 3.22 (s, 3 H, CH_3).

A solution of **46f** (684 mg, 1.42 mmol) in THF (50 mL) was hydrogenated over Raney nickel (0.5 g) at 50.7 psi for 22 h. The mixture was filtered through Celite, and the solution was stripped rigorously to dryness to give 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-(3,6,9-trioxadecanoxy)quinazoline (**50f**) (654 mg, 95% corrected), contaminated with 17 mol % THF and 9 mol % BHT stabilizer, as a gray-green waxy solid that was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 9.44 (br s, 1 H, NH), 8.41 (s, 1 H, H-2), 8.23 (dd, 1 H, J = 2.6, 6.7 Hz, H-2′), 7.84 (ddd, 1 H, J = 2.6, 4.1, 9.1 Hz, H-6′), 7.44 (s, 1 H, H-5), 7.43 (t, 1 H, J = 9.2 Hz, H-5′), 7.16 (s, 1 H, H-8), 5.36 (br s, 2 H, NH_2), 4.33 (t, 2 H, J = 4.5 Hz, $ArOCH_2$), 3.91 (t, 2 H, J = 4.5 Hz, $ArOCH_2CH_2$), 3.68, 3.60 (dds, 2 H, 2 H, J = 3.6, 6.4 Hz, OCH_2s), 3.56, 3.46 (dds, 2 H, 2 H, J = 3.7, 5.7 Hz, OCH_2s), 3.27 (s, 3 H, CH_3).

Solid **50f** was added in one portion to a solution of the anhydride made by adding isobutyl chloroformate (65 mL, 0.5 mmol) dropwise to a stirred solution of acrylic acid (42 mg, 0.58 mmol) and Et_3N (101 mg, 1.0 mmol) in THF (2.5 mL) at 0 °C under N_2, and keeping for 10 min. After a further 20 min at 0 °C, the reaction mixture was applied directly to preparative TLC plates (silica, two 20 × 20 × 0.2 cm) and eluted once with CH_2Cl_2/MeOH (19:1). The major band (R_f 0.28) was extracted with MeOH/CH_2Cl_2, and the solvent was removed under reduced pressure to give **19** (67 mg, 49%): mp 131−132 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.83 (br s, 1 H, NH), 9.63 (br s, 1 H, NH), 8.91 (s, 1 H, H-5), 8.54 (s, 1 H, H-2), 8.14 (dd, 1 H, J = 2.7, 6.8 Hz, H-2′), 7.80 (ddd, 1 H, J = 2.7, 4.4, 9.0 Hz, H-6′), 7.43 (t, 1 H, J = 9.1 Hz, H-5′), 7.34 (s, 1 H, H-8), 6.72 (dd, 1 H, J = 17.0, 10.4 Hz, CH=CH_2), 6.32 (dd, 1 H, J = 1.9, 17.1 Hz, CH=CH_2), 5.83 (dd, 1 H, J = 1.9, 10.2 Hz, CH=CH_2), 4.38 (t, 2 H, J = 4.5 Hz, Ar OCH_2), 3.89 (t, 2 H, J = 4.5 Hz, $ArOCH_2CH_2$), 3.64, 3.54 (dds, 2 H, 2 H, J = 5.4, 6.3 Hz, OCH_2s), 3.50, 3.41 (dds, 2 H, 2 H, J = 4.9, 6.4 Hz, OCH_2s), 3.21 (s, 3 H, OCH_3); MS (APCI) 507 (57, $^{37}ClMH^+$), 505 (100, $^{35}ClMH^+$). Anal. ($C_{24}H_{26}ClFN_4O_5 \cdot 2H_2O$) C, H, N.

***N*-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[(1*E*)-4-(4-morpholinyl)-1-butenyl]pyrido[3,2-*d*]pyrimidin-6-yl]-acrylamide (21). Example of the Method of Scheme 3.** A solution of 6-chloro-3-nitropyridine-2-carboxamide (**51**)[9] (1.00 g, 4.96 mmol) in EtOH (60 mL) and concentrated ammonia (30 mL) was sealed and heated at 100 °C for 20 h. The mixture was then cooled, concentrated under reduced pressure, and chromatographed on silica gel. Eluants from EtOAc to MeOH/EtOAc (1:4) gave 6-amino-3-nitropyridine-2-carboxamide (**52**) (658 mg, 73%): mp (EtOAc/MeOH) 219−226 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 8.11 (d, J = 9.2 Hz, 1 H, H-4), 7.84 (s, 1 H, $CONH_2$), 7.57 (s, 2 H, NH_2), 7.52 (s, 1 H, $CONH_2$), 6.50 (d, J = 9.2 Hz, 1 H, H-5). Anal. ($C_6H_6N_4O_3$) C, H, N.

A suspension **52** (0.60 g, 3.29 mmol) in glacial AcOH (70 mL) was heated until homogeneous, then treated with solid KOAc (0.65 g, 6.59 mmol) and allowed to cool to 50 °C. Br_2 (0.34 mL, 6.59 mmol) was then added dropwise, and the reaction was allowed to cool and stirred at room temperature for 1.5 h. The resultant suspension was concentrated under reduced pressure, then diluted with water and basified with solid $NaHCO_3$. The aqueous layer was extracted with EtOAc, and this extract was evaporated and the residue chromatographed on silica gel, eluting with EtOAc to MeOH/EtOAc (1:4), to give 6-amino-5-bromo-3-nitropyridine-2-carboxamide (**53**) (0.72 g, 84%): mp (EtOAc) 250−252 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.47 (s, 1 H, H-4), 8.20−7.30 (br s, 2 H, NH_2), 7.89 (s, 1 H, $CONH_2$), 7.64 (s, 1 H, $CONH_2$). Anal. ($C_6H_5BrN_4O_3$) C, H, N, Br.

Iron dust reduction of **53** (14.44 g, 53.3 mmol) as described previously gave crude 5-bromo-3,6-diaminopyridine-2-carboxamide (**54**). This was not characterized further, but was suspended in triethyl orthoformate (300 mL) and heated at reflux for 3 h. The resulting suspension was concentrated under reduced pressure and diluted with hexane (200 mL). The resulting precipitate was collected, washed with hexane and dried, then dissolved in 1 N NaOH (600 mL) (heating) and the solution stirred at room temperature for 2 h. Acidification with saturated NH_4Cl precipitated 6-amino-7-bromopyrido[3,2-*d*]pyrimidin-4(3*H*)-one (**55**) (8.3 g, 62%): mp (AcOH) 300−306 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 12.24 (br s, 1 H, NH), 8.12 (s, 1 H, aromatic), 7.88 (s, 1 H, aromatic), 6.91 (br s, 2 H, NH_2). Anal. ($C_7H_5BrN_4O$) C, H, N.

A suspension of **55** (3.96 g, 16.4 mmol) in 50% HBF_4 (80 mL) was cooled to −10 °C, and $NaNO_2$ (2.27 g, 32.86 mmol) was added to this suspension in portions over 4 h. The resulting mixture was then kept at −20 °C for 18 h before being warmed to −10 °C and neutralized by the slow addition of solid Na_2CO_3 (keeping the reaction temperature below −5 °C). The precipitate was filtered, washed with water and dried, then suspended in hot DMF and filtered through a pad of Celite which was washed with further hot DMF. The combined filtrates were evaporated to dryness under reduced pressure and chromatographed on silica gel. Gradient elution from MeOH/CH_2Cl_2 (1:9) to MeOH/CH_2Cl_2 (1:4) gave 7-bromo-6-fluoropyrido[3,2-*d*]pyrimidin-4(3*H*)-one (**56**) (2.15 g, 54%): mp (MeOH/H_2O) 301−305 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 12.81 (br s, 1 H, NH), 8.69 (d, J_{H-F} = 8.1 Hz, 1 H, H-8), 8.21 (s, 1 H, H-2). Anal. ($C_7H_3BrFN_3O$) C, H, N, F.

A suspension of **56** (70 mg, 0.29 mmol) in $POCl_3$ (15 mL) was heated at reflux for 20 min (when all material had dissolved), and then for a further 1.5 h. Excess $POCl_3$ was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2, washed several times with saturated $NaHCO_3$ and brine and dried (Na_2SO_4). Evaporation under reduced pressure gave crude 7-bromo-4-chloro-6-fluoropyrido[3,2-*d*]-pyrimidine, which was dissolved in *i*-PrOH (15 mL) and added to a solution of 3-chloro-4-fluoroaniline (84 mg, 0.57 mmol) in *i*-PrOH (20 mL) containing 1 drop of concentrated HCl. The resulting solution was heated under reflux for 5 min, and then at 60 °C for 30 min. The volume was then reduced to approximately 10 mL by evaporation under reduced pressure, and saturated aqueous $NaHCO_3$ was added. The resulting precipitate was collected by filtration, washed with water and dried under vacuum to give 7-bromo-4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-fluoropyrido[3,2-*d*]pyrimidine (**57**) (96 mg, 90%): mp (EtOAc) 249−251 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.37 (s, 1 H, NH), 8.84 (d, J_{H-F} = 8.3 Hz, 1 H, H-8), 8.72 (d, J = 1.9 Hz, 1 H, H-2), 8.32 (m, 1 H, H-2′), 7.98 (m, 1 H, H-6′), 7.46 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′). Anal. ($C_{13}H_6BrClF_2N_4$) C, H, N.

A stirred solution of 3-butyn-1-ol (6.00 g, 85.6 mmol) in CH_2Cl_2 (200 mL) at 0 °C was treated with Et_3N (256.8 mmol, 35.8 mL) followed by MsCl (111.3 mmol, 8.6 mL) dropwise. After stirring the reaction mixture for 30 min, morpholine (119.8 mmol, 10.5 mL) was added and the reaction mixture was allowed to warm to room temperature for 16 h. Further morpholine (10.5 mL) was then added, and the reaction was heated at reflux for 8 h. The solution was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting crude oil was filtered through a short column of silica gel, eluting with EtOAc/hexane (1:1), and the eluate, after the removal of solvent, was distilled to give 4-(3-butynyl)morpholine (**58**) (6.47 g, 54%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.72 (t, J = 4.7 Hz, 4 H, morpholino methylene), 2.59 (t, J = 7.8 Hz, 2 H, H-4), 2.49 (t, J = 4.7 Hz, 4 H, morpholino methylene), 2.38 (m, 2 H, H-3), 1.98 (s, 1H, H-1).

To neat degassed **58** (3.64 g, 26.2 mmol) under N_2 was added AIBN (catalytic, 50 mg) followed by tributyltin hydride (26.2 mmol, 7.04 mL). The reaction mixture was heated to 90 °C with adequate venting (vigorous initial reaction) and kept at 90 °C for a further 16 h. The cooled mixture was diluted with hexane and chromatographed directly on silica gel, using gradient elution from hexane to EtOAc/hexane (2:3), to give 4-[(3*E*)-4-(tributylstannyl)-3-butenyl]morpholine (**59**) (7.76 g, 69%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.94 (m, 2 H, CH=CH), 3.71 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.46 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.42 (partially obscured, m, 2 H, NCH_2CH_2CH), 2.33 (m, 2 H, NCH_2CH_2CH),

1.48 (m, 6 H, $Sn(CH_2CH_2CH_2CH_3)_3$), 1.30 (m, 6 H, $Sn(CH_2CH_2CH_2CH_3)_3$), 0.89m (partially obscured t, $J = 7.2$ Hz, 9 H, $Sn(CH_2CH_2CH_2CH_3)_3$), 0.87 (partially obscured m, 6 H, $Sn(CH_2CH_2CH_2CH_3)_3$); ^{13}C NMR δ 146.6, 129.2, 67.0 (×2), 58.4, 53.7 (×2), 35.2, 29.1 (×3), 13.7 (×3), 9.4 (×3).

The above stannane **59** (19.29 mmol, 8.30 g) was added to a degassed solution of **57** (4.78 g, 12.86 mmol) and catalytic tetrakistriphenylphosphinepalladium(0) (1.29 mmol, 1.49 g), in DMF (100 mL) under nitrogen. The reaction mixture was heated to 100 °C for 75 min (reaction complete by TLC), then diluted with water and extracted with EtOAc. The combined organic extracts were washed with water and dried (Na_2SO_4), then evaporated under reduced pressure. Chromatography on silica gel, eluting with EtOAc to MeOH/EtOAc (1:4), gave 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-fluoro-7-[(1*E*)-4-(4-morpholinyl)-1-butenyl]pyrido[3,2-*d*]pyrimidine (**60**) (3.98 g, 72%): mp (Et_2O/hexane) 189−190 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.21 (s, 1 H, NH), 8.70 (s, 1 H, H-2), 8.48 (d, $J_{H-F} = 9.2$ Hz, 1 H, H-8), 8.35 (m, 1 H, H-2′), 7.99 (m, 1 H, H-6′), 7.45 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 6.85 (m, 1 H, CH_2CH═CH), 6.66 (d, $J = 16.0$ Hz, 1 H, CH_2CH═C*H*), 3.59 (t, $J = 4.5$ Hz, 4 H, morpholino methylene), 2.50 (br s, 4 H, CH_2CH_2CH═CH), 2.42 (t, $J = 4.5$ Hz, 4 H, morpholino methylene). Anal. ($C_{21}H_{20}ClF_2N_5O$) C, H, N.

A suspension of **60** (325 mg, 0.753 mmol) in *i*-PrOH (60 mL) was saturated with NH_3(g), then sealed in a pressure vessel and heated at 100 °C for 20 h. The resulting solution was cooled and evaporated to dryness under reduced pressure, then chromatographed on silica gel, eluting with MeOH/EtOAc (1:8), to give 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[(1*E*)-4-(4-morpholinyl)-1-butenyl]pyrido[3,2-*d*]pyrimidine (**61**) (316 mg, 98%): mp (EtOAc) 157−158 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.36 (s, 1 H, NH), 8.44 (s, 1 H, H-2), 8.41 (m, 1 H, H-2′), 7.88 (s, 1 H, H-8), 7.86 (m, 1 H, H-6′), 7.43 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 6.69 (partially obscured d, $J = 15.5$ Hz, 1 H, CH_2CH═C*H*), 6.67 (s, 2 H, NH_2), 6.49 (dt, $J = 15.5$, 6.7 Hz, 1 H, CH_2CH═CH), 3.59 (t, $J = 4.6$ Hz, 4 H, morpholino methylene), 2.48−2.43 (m, 4 H, CH_2CH_2CH═CH), 2.42 (br t, $J = 4.6$ Hz, 4 H, morpholino methylene). Anal. ($C_{21}H_{22}ClFN_6O$) C, H, N.

Acrylic acid (36.0 mmol, 2.54 mL) was added to a solution of Et_3N (52.9 mmol, 7.44 mL) in dry THF (100 mL) at 0 °C under N_2. This mixture was then treated dropwise with 2,4,6-trichlorobenzoyl chloride (31.7 mmol, 4.90 mL), and the resulting suspension was stirred for 15 min. A solution of **61** (907 mg, 2.11 mmol) and DMAP (0.42 mmol, 51 mg) in dry THF (60 mL) under N_2 was then added via cannula. The resulting mixture was stirred at 0 °C for a further 15 min before being warmed to room temperature. After 2 h (when TLC showed the reaction was complete), the mixture was diluted with water and extracted with EtOAc. The combined organic extracts were washed with water and dried (Na_2SO_4), then concentrated under reduced pressure and filtered through a plug of alumina in EtOAc to remove yellow polymeric baseline material. The crude residue was then stirred overnight in aqueous MeOH (10%, 80 mL) to hydrolyze any bisacyl material. The solvent was then partially removed under reduced pressure, and the residue was diluted with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc/DCM/MeOH (60:25:15), to give **21** (340 mg, 33%): mp (EtOAc/MeOH/Et_2O) 190−193 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.65 (s, 1 H, CONH), 10.00 (s, 1 H, NH), 8.69 (s, 1 H, H-2), 8.39 (m, 1 H, H-2′), 8.37 (s, 1 H, H-8), 7.97 (m, 1 H, H-6′), 7.45 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 6.67 (dt, $J = 15.9$, 6.5 Hz, 1 H, CH_2CH═CH), 6.57 (dd, $J = 17.1$, 10.2 Hz, 1 H, $COCH$═CH_2), 6.52 (d, $J = 15.9$ Hz, 1 H, CH_2CH═C*H*), 6.34 (dd, $J = 17.1$, 1.7 Hz, 1 H, $COCH$═C*H*$_2$), 5.86 (dd, $J = 10.2$, 1.7 Hz, 1 H, $COCH$═C*H*$_2$), 3.58 (t, $J = 4.6$ Hz, 4 H, morpholino methylene), 2.44−2.37 (m, 8 H, CH_2CH_2CH═CH, morpholino methylene). HRMS (FAB) M + 1 Required for $C_{24}H_{24}{}^{35}ClFN_6O_2$: 483.17114. Found: 483.17136.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-[4-(4-morpholinyl)butyl]pyrido[3,2-*d*]pyrimidin-6-yl]acrylamide (22).** A solution of **60** (3.60 g, 8.34 mmol) in THF/MeOH (2:1, 400 mL) was hydrogenated over $PtO_2 \cdot xH_2O$ at 60 psi for 18 h before being filtered through Celite and concentrated under reduced pressure. Chromatography on a short column of alumina, eluting with MeOH/EtOAc (1:9), gave 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-fluoro-7-[4-(4-morpholinyl)butyl]pyrido-[3,2-*d*]pyrimidine (**62**) (3.40 g, 94%): mp (EtOAc) 147−148 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.21 (s, 1 H, NH), 8.70 (d, $J = 1.6$ Hz, 1 H, H-2), 8.36 (m, 1 H, H-2′), 8.28 (d, $J_{H-F} = 9.1$ Hz, 1 H, H-8), 7.99 (m, 1 H, H-6′), 7.45 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 3.55 (t, $J = 4.6$ Hz, 4 H, morpholino methylene), 2.84 (t, $J = 7.5$ Hz, 2 H, CH_2Ar), 2.31 (m, 6 H, morpholino methylene, $NCH_2CH_2CH_2CH_2$), 1.71 (quintet, $J = 7.5$ Hz, 2 H, $NCH_2CH_2CH_2CH_2Ar$), 1.50 (quintet, $J = 7.5$ Hz, 2 H, $NCH_2CH_2CH_2CH_2Ar$). Anal. ($C_{21}H_{22}ClF_2N_5O$) C, H, N.

A suspension of **62** (1.83 g, 4.22 mmol) in *i*-PrOH (240 mL) was treated with NH_3(g) as above to give 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[4-(4-morpholinyl)butyl]pyrido-[3,2-*d*]pyrimidine (**63**) (1.65 g, 91%): mp (MeOH) 182−183 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.33 (s, 1 H, NH), 8.44 (s, 1 H, H-2), 8.41 (ddd, $J_{H-F} = 6.9$ Hz, $J = 2.6$, 2.6 Hz, 1 H, H-2′), 7.85 (m, 1 H, H-6′), 7.67 (s, 1 H, H-8), 7.43 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 6.64 (s, 2 H, NH_2), 3.57 (t, $J = 4.6$ Hz, 4 H, morpholino methylene), 2.64 (t, $J = 7.4$ Hz, 2 H, CH_2Ar), 2.33 (m, 6 H, morpholino methylene, $NCH_2CH_2CH_2CH_2$), 1.64 (quintet, $J = 7.4$ Hz, 2 H, $NCH_2CH_2CH_2CH_2Ar$), 1.53 (quintet, $J = 7.4$ Hz, 2 H, $NCH_2CH_2CH_2CH_2Ar$). Anal. ($C_{21}H_{24}ClFN_6O$) C, H, N.

To a solution of **63** (1.65 g, 3.82 mmol) in DMA/THF (1:1, 100 mL) at 0 °C were added 1-hydroxybenzotriazole (3.82 mmol, 516 mg), triethylamine (57.3 mmol, 4.1 mL), acrylic acid (22.9 mmol, 1.58 mL) and EDCI·HCl (22.9 mmol, 4.40 g). The reaction mixture was stirred at 0 °C under N_2 for 30 min, then allowed to warm to room temperature. Additional acrylic acid (260 μL) and EDCI·HCl (730 mg) were added to the suspension twice weekly. After 6 weeks (despite considerable starting material remaining), the reaction mixture was diluted with water, basified with saturated aqueous $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were washed with brine and dried (Na_2SO_4), then evaporated under reduced pressure. Chromatography (2×) on silica gel, eluting with EtOAc/CH_2Cl_2/MeOH (7:2:1) gave a fraction (approximately 400 mg) enriched in the desired product. Reverse-phase preparative layer chromatography of this, eluting with MeOH/H_2O/cNH_3 (93:5:2) (running each plate 3×) gave, at highest R_f, **22** (155 mg, 8%, 28% based on recovered starting material): mp (EtOAc) 180−182 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.57 (s, 1 H, CONH), 10.00 (s, 1 H, NH), 8.69 (d, $J = 1.6$ Hz, 1 H, H-2), 8.37 (m, 1 H, H-2′), 8.13 (s, 1 H, H-8), 7.96 (m, 1 H, H-6′), 7.45 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 6.56 (dd, $J = 17.0$, 10.2 Hz, 1 H, C*H*═CH_2), 6.33 (dd, $J = 17.0$, 1.8 Hz, 1 H, CH═C*H*$_2$), 5.85 (dd, $J = 10.2$, 1.8 Hz, 1 H, CH═C*H*$_2$), 3.54 (t, $J = 4.6$ Hz, 4 H, morpholino methylene), 2.78 (t, $J = 7.5$ Hz, 2 H, CH_2Ar), 2.27 (m, 6 H, morpholino methylene, $NCH_2CH_2CH_2CH_2$), 1.66 (quintet, $J = 7.5$ Hz, 2 H, $NCH_2CH_2CH_2CH_2Ar$), 1.44 (quintet, $J = 7.5$ Hz, 2 H, $NCH_2CH_2CH_2CH_2Ar$). Anal. ($C_{24}H_{26}ClFN_6O_2 \cdot 0.5H_2O$) C, H, N.

Starting material **63** (1.16 g combined weight from column and preparative layer chromatography, 70%) was also recovered: ^{1}H NMR identical to an authentic sample.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-methoxypyrido[3,2-*d*]pyrimidin-6-yl]acrylamide (23). Example of the Method of Scheme 4.** A solution of 2-bromo-5-methoxy-6-(methylthio)-3-nitropyridine[18] (**64**) (7.70 g, 27.6 mmol) in NMP (50 mL) was added to a solution of CuCN (4.94 g, 55.2 mmol) in NMP (200 mL) at 170 °C. The resultant mixture was heated at 170 °C for 10 min, then cooled and poured into ice water (2 L). The resulting precipitate was collected by filtration, washed with water, suspended in boiling EtOAc (400 mL) and filtered hot through a pad of Celite. The Celite was washed with EtOAc, and the combined filtrates were dried (Na_2SO_4) and evaporated to give 2-cyano-5-methoxy-6-(methylthio)-3-

nitropyridine (**65**) (5.90 g, 95%): mp (EtOAc/hexane) 168−170 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.02 (s, 1 H, H-4), 4.10 (s, 3 H, OCH_3), 2.58 (s, 3 H, SCH_3). Anal. ($C_8H_7N_3O_3S$) C, H, N.

A solution of **65** (5.19 g, 23.0 mmol) in 90% H_2SO_4 (80 mL) was warmed at 50 °C for 1.5 h before being poured onto ice (1 L) and extracted with EtOAc. The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, then dried (Na_2SO_4). Evaporation gave a residue that was chromatographed on silica gel, eluting with EtOAc/hexane (1:1), to give 5-methoxy-6-(methylthio)-3-nitropyridine-2-carboxamide (**66**) (0.80 g, 14%): mp (Et_2O/hexane) 215−217 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.04 (s, 1 H, $CONH_2$), 7.84 (partially obscured s, 1 H, $CONH_2$), 7.83 (s, 1 H, H-4), 3.99 (s, 3 H, OCH_3), 2.58 (s, 3 H, SCH_3). Anal. ($C_8H_9N_3O_4S$) C, H, N.

A solution of **66** (0.57 g, 2.34 mmol) in EtOAc/MeOH (1:1, 150 mL) was hydrogenated over Pd−C at 60 psi for 16 h, before fresh catalyst was added and the hydrogenation continued for a further 7 h. The solution was then filtered through Celite and evaporated to give crude 3-amino-5-methoxy-6-(methylthio)pyridine-2-carboxamide (**67**), which was suspended in neat triethyl orthoformate (30 mL) and heated under reflux for 8 h. The reaction mixture was concentrated under reduced pressure, hexane was added, and the resultant precipitate was collected, washed with hexane and dried to give 7-methoxy-6-(methylthio)pyrido[3,2-*d*]pyrimidin-4(3*H*)-one (**68**) (422 mg, 81%): mp (MeOH) 279−281 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.44 (s, 1 H, NH), 8.08 (s, 1 H, H-2), 7.37 (s, 1 H, H-8), 4.00 (s, 3 H, OCH_3), 2.55 (s, 3 H, SCH_3). Anal. ($C_9H_9N_3O_2S$) C, H, N.

Iron dust reduction of **65** (22.0 g, 97.7 mmol) as above gave crude 3-amino-2-cyano-5-methoxy-6-(methylthio)pyridine (**69**) which was filtered through a short column of silica gel, eluting with MeOH/EtOAc (1:9) to remove baseline impurities. The amine **69** was then added in portions over 1 h to a refluxing solution of 88% HCO_2H (200 mL) and concentrated H_2SO_4 (3.6 mL). The reaction was refluxed for 16 h, then cooled to room temperature and poured into ice water (2 L). The precipitate was filtered, washed with water, CH_2Cl_2/Et_2O (1:1) and then Et_2O and dried to give **68** (17.98 g, 82%): ^{1}H NMR identical to an authentic sample.

Activation of **68** (0.52 g, 2.33 mmol) in $POCl_3$ (20 mL) and subsequent reaction with 3-chloro-4-fluoroaniline (0.68 g, 4.66 mmol) as above gave, after chromatography on silica gel, eluting with EtOAc/CH_2Cl_2 (1:1), 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-methoxy-6-(methylthio)pyrido[3,2-*d*]pyrimidine (**70a**) (0.63 g, 77%): mp (CH_2Cl_2/Et_2O) 234−236 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.54 (s, 1 H, NH), 8.59 (s, 1 H, H-2), 8.26 (m, 1 H, H-2′), 7.93 (m, 1 H, H-6′), 7.47 (m, 2 H, H-8, H-5′), 4.05 (s, 3 H, OCH_3), 2.75 (s, 3 H, SCH_3). Anal. ($C_{15}H_{12}ClFN_4OS$) C, H, N.

A solution of **70a** (0.62 g, 1.77 mmol) in CH_2Cl_2 (350 mL) was treated with *m*-CPBA (1.94 mmol, 0.45 g of 75%) for 1 h at room temperature, then washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated under reduced pressure. Addition of hexane to the concentrate gave a precipitate that was collected and washed with hexane to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-(methanesulfinyl)-7-methoxypyrido[3,2-*d*]pyrimidine (**71a**) (0.63 g, 97%): mp (CH_2Cl_2/Et_2O) 222−224 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.17 (s, 1 H, NH), 8.69 (s, 1 H, H-2), 8.28 (dd, J_{H-F} = 6.8 Hz, J = 2.5 Hz, 1 H, H-2′), 7.92 (m, 1 H, H-6′), 7.83 (s, 1 H, H-8), 7.48 (dd, J_{H-F} = 9.0 Hz, J = 9.0 Hz, 1 H, H-5′), 4.09 (s, 3 H, OCH_3), 3.06 (s, 3 H, S(O)CH_3). Anal. ($C_{15}H_{12}ClFN_4O_2S$) C, H, N.

A suspension of **71a** (0.63 g, 1.72 mmol) in *i*-PrOH (100 mL) was reacted with NH_3(g) as above to give, after column chromatography on silica gel and elution with CH_2Cl_2/EtOAc (1:1) to MeOH/ CH_2Cl_2/EtOAc (2:48:50), 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-methoxypyrido[3,2-*d*]pyrimidine (**72a**) (0.23 g, 42%): mp (CH_2Cl_2/Et_2O) 258−260 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.23 (s, 1 H, NH), 8.44 (s, 1 H, H-2), 8.39 (m, 1 H, H-2′), 7.84 (m, 1 H, H-6′), 7.41 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 7.28 (s, 1 H, H-8), 6.68 (s, 2 H, NH_2), 3.99 (s, 3 H, OCH_3). Anal. ($C_{14}H_{11}ClFN_5O$) C, H, N.

A solution of **72a** (0.25 g, 0.782 mmol) in THF was reacted with the mixed anhydride of 2,4,6-trichlorobenzoyl chloride and acrylic acid as described above. Chromatography on silica gel, with gradient elution from CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (1:4:5), gave **23** (123 mg, 42%): mp (EtOAc/Et_2O) 226−228 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.39 (s, 1 H, CONH), 9.69 (s, 1 H, NH), 8.65 (s, 1 H, H-2), 8.36 (m, 1 H, H-2′), 7.89 (m, 1 H, H-6′), 7.69 (s, 1 H, H-8), 7.45 (dd, J_{H-F} = 9.0 Hz, J = 9.0 Hz, 1 H, H-5′), 6.66 (dd, J = 17.1, 10.2 Hz, 1 H, C*H*=CH_2), 6.33 (dd, J = 17.1, 1.9 Hz, 1 H, CH=C*H*$_2$), 5.84 (dd, J = 10.2, 1.9 Hz, 1 H, CH=C*H*$_2$), 4.04 (s, 3 H, OCH_3). Anal. ($C_{17}H_{13}ClFN_5O_2$) C, H, N.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-(2-methoxyethoxy)pyrido[3,2-*d*]pyrimidin-6-yl]acrylamide (24). Example of the Method of Scheme 5.** Anhydrous pyridine hydrochloride was prepared by adding concentrated HCl (37%, 148 mL) to a solution of pyridine (150 mL) in CH_2Cl_2 (250 mL) at 0 °C, followed by concentration to dryness under reduced pressure. This was then heated to 200 °C under N_2, and solid **70a** (10.5 g, 29.9 mmol) was then added. Once the solid had gone into solution (approximately 3 min) the reaction was heated at 200−210 °C for a further 5 min before being cooled to approximately 100 °C and diluted with water (500 mL). The resulting precipitate was collected by filtration and washed with water, then dissolved in DMSO (150 mL) (heating to 90 °C) and treated with activated charcoal for 10 min. The solution was filtered through Celite, the Celite was washed with DMSO (50 mL), and the combined filtrates were diluted with water (300 mL). The precipitate was collected, washed with water and Et_2O and dried to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-hydroxy-6-(methylthio)pyrido-[3,2-*d*]pyrimidine (**73**) (8.05 g, 80%): mp (MeOH) 290−300 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.82 (br s, 1 H, OH), 9.51 (s, 1 H, NH), 8.53 (s, 1 H, H-2), 8.26 (m, 1 H, H-2′), 7.92 (m, 1 H, H-6′), 7.46 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 7.18 (s, 1 H, H-8), 2.72 (s, 3 H, SCH_3). Anal. ($C_{14}H_{10}ClFN_4OS$) C, H, N.

A suspension of **73** (400 mg, 1.19 mmol) in DMF (15 mL) was heated briefly to 100 °C, and the resultant solution was cooled to 60 °C and treated successively with K_2CO_3 (1.15 g, 8.32 mmol) and 2-bromoethyl methyl ether (558 μL, 5.94 mmol). The resulting mixture was heated at 60 °C for 2 h, then diluted with water and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated, and the residue was chromatographed on silica gel, eluting with EtOAc/CH_2Cl_2 (1:1) to EtOAc/CH_2Cl_2/MeOH (10:9:1), to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-(2-methoxyethoxy)-6-(methylthio)pyrido-[3,2-*d*]pyrimidine (**70b**) (342 mg, 73%): mp (MeOH/Et_2O) 169−171 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.53 (s, 1 H, NH), 8.58 (s, 1 H, H-2), 8.27 (m, 1 H, H-2′), 7.93 (m, 1 H, H-6′), 7.49 (s, 1 H, H-8), 7.46 (partially obscured dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 4.40 (t, J = 4.3 Hz, 2 H, C*H*$_2$CH$_2$OCH$_3$), 3.76 (t, J = 4.3 Hz, 2 H, CH$_2$C*H*$_2$OCH$_3$), 3.37 (s, 3 H, OCH_3), 2.74 (s, 3 H, SCH_3). Anal. ($C_{17}H_{16}ClFN_4O_2S$) C, H, N.

A solution of **70b** (242 mg, 0.613 mmol) in CH_2Cl_2 (70 mL) was oxidized with *m*-CPBA as above to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-(methanesulfinyl)-7-(2-methoxyethoxy)-pyrido[3,2-*d*]pyrimidine (**71b**) (146 mg, 58%): mp (MeOH/EtOAc) 237−240 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.50 (s, 1 H, NH), 8.70 (s, 1 H, H-2), 8.32 (dd, J_{H-F} = 6.9 Hz, J = 2.7 Hz, 1 H, H-2′), 7.95 (m, 1 H, H-6′), 7.85 (s, 1 H, H-8), 7.48 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 4.48 (m, 2 H, C*H*$_2$CH$_2$OCH$_3$), 3.76 (t, J = 4.3 Hz, 2 H, CH$_2$C*H*$_2$OCH$_3$), 3.37 (s, 3 H, OCH_3), 3.02 (s, 3 H, S(O)CH_3). Anal. ($C_{17}H_{16}ClFN_4O_3S\cdot0.5H_2O$) C, H, N.

A suspension of **71b** (0.30 g, 0.73 mmol) in *i*-PrOH (80 mL) was reacted with NH_3(g) as above to give 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-(2-methoxyethoxy)pyrido[3,2-*d*]pyrimidine (**72b**) (251 mg, 94%): mp (EtOAc/Et_2O) 195−197 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.24 (s, 1 H, NH), 8.44 (s, 1 H, H-2), 8.40 (dd, J_{H-F} = 6.9 Hz, J = 2.7 Hz, 1 H, H-2′), 7.85 (m, 1 H, H-6′), 7.41 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 7.31 (s, 1 H, H-8), 6.61 (br s, 2 H, NH_2), 4.34 (m, 2 H, C*H*$_2$CH$_2$OCH$_3$), 3.78 (m, 2 H, CH$_2$C*H*$_2$OCH$_3$), 3.35 (s, 3 H, OCH_3). Anal. ($C_{16}H_{15}ClFN_5O_2$) C, H, N.

A solution of **72b** (0.24 g, 0.66 mmol) in THF (35 mL) was reacted with the mixed anhydride of 2,4,6-trichlorobenzoyl chloride/acrylic acid as described above. Chromatography on silica gel with gradient elution from CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (1:4:5) gave **24** (87 mg, 32%): mp (EtOAc/Et_2O) 205−206 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.31 (s, 1 H, CONH), 9.72 (s, 1 H, NH), 8.64 (s, 1 H, H-2), 8.35 (m, 1 H, H-2′), 7.91 (m, 1 H, H-6′), 7.73 (s, 1 H, H-8), 7.45 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 6.66 (dd, J = 17.0, 10.2 Hz, 1 H, C*H*═CH_2), 6.33 (dd, J = 17.0, 1.9 Hz, 1 H, CH═CH_2), 5.83 (dd, J = 10.2, 1.9 Hz, 1 H, CH═CH_2), 4.38 (m, 2 H, CH_2CH_2-OCH_3), 3.75 (m, 2 H, C$H_2CH_2OCH_3$), 3.35 (s, 3 H, OCH_3). Anal. ($C_{19}H_{17}ClFN_5O_3$) C, H, N.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]pyrido[3,2-*d*]pyrimidin-6-yl]-acrylamide (25).** A suspension of **73** (296 mg, 0.88 mmol) in THF/DMF (4:1, 25 mL) was treated successively with K_2CO_3 (1.2 g, 8.80 mmol) and 3-bromopropan-1-ol (397 μL, 4.40 mmol). The reaction mixture was heated at reflux for 1.25 h, then diluted with water and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. The residue was dry-loaded on silica gel and chromatographed, eluting with EtOAc/CH_2Cl_2 (1:1) to EtOAc/CH_2Cl_2/MeOH (10:9:1), to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-(3-hydroxypropoxy)-6-(methylthio)pyrido-[3,2-*d*]pyrimidine (**74**) (215 mg, 62%): mp (MeOH/CH_2Cl_2) 212−214 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.54 (s, 1 H, NH), 8.58 (s, 1 H, H-2), 8.27 (dd, J_{H-F} = 6.8 Hz, J = 2.5 Hz, 1 H, H-2′), 7.93 (m, 1 H, H-6′), 7.47 (partially obscured dd, J_{H-F} = 9.2 Hz, J = 9.2 Hz, 1 H, H-5′), 7.46 (partially obscured s, 1 H, H-8), 4.63 (t, J = 5.1 Hz, 1 H, OH), 4.33 (t, J = 6.1 Hz, 2 H, CH_2OAr), 3.63 (m, 2 H, CH_2OH), 2.74 (s, 3 H, SCH_3) 1.95 (quintet, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2OH$). Anal. ($C_{17}H_{16}ClFN_4O_2S\cdot0.5H_2O$) C, H, N.

A suspension of **74** (1.65 g, 4.18 mmol) in THF/DMF (3:1, 130 mL) was warmed to give a solution which was then cooled to 0 °C before Et_3N (12.5 mmol, 1.75 mL) and MsCl (4.60 mmol, 0.355 mL) were added. After 1 h the reaction mixture was concentrated under reduced pressure at <30 °C, then diluted with saturated $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to give the crude mesylate. This was immediately dissolved in THF (100 mL), treated with excess morpholine (4.0 mL) and heated at 50 °C for 4 days. The mixture was then concentrated, diluted with ammonia and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. The crude product was slurried in EtOAc/hexane (1:2), filtered, and washed with Et_2O to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-(methylthio)-7-[3-(4-morpholinyl)propoxy]-pyrido[3,2-*d*]pyrimidine (**70c**) (1.46 g, 75%): mp (EtOAc) 196−198 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.54 (s, 1 H, NH), 8.59 (d, J = 1.4 Hz, 1 H, H-2), 8.28 (m, 1 H, H-2′), 7.93 (m, 1 H, H-6′), 7.47 (m, 2 H, H-8, H-5′), 4.32 (t, J = 6.1 Hz, 2 H, CH_2OAr), 3.58 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.74 (s, 3 H, SCH_3), 2.44 (partially obscured m, 2 H, N$CH_2CH_2CH_2OAr$), 2.39 (br m, 4 H, morpholino methylene), 1.97 (quintet, J = 6.1 Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{21}H_{23}ClFN_5O_2S\cdot H_2O$) C, H. N.

A suspension of **70c** (168 mg, 0.362 mmol) in 1 N HCl (100 mL) was warmed to 40 °C to give a solution which was then cooled to 0 °C, treated with added $NaIO_4$ (0.398 mmol, 85 mg) and kept at 4 °C for 48 h. It was then warmed to 40 °C to dissolve precipitated starting material and cooled to 0 °C when additional $NaIO_4$ (40 mg) was added. After a further 24 h at 4 °C the reaction was basified by the addition of concentrated ammonia and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Chromatography of the residue, eluting with MeOH/CH_2Cl_2/EtOAc (3:6:10), gave 4-[*N*-(3-chloro-4-fluorophenyl)-amino]-6-(methanesulfinyl)-7-[3-(4-morpholinyl)propoxy]-pyrido[3,2-*d*]pyrimidine (**71c**) (163 mg, 94%): mp (MeOH/CH_2Cl_2/hexane) 160−162 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.13 (s, 1 H, NH), 8.69 (d, J = 2.0 Hz, 1 H, H-2), 8.28 (ddd, J_{H-F} = 6.7 Hz, J = 6.7, 2.6 Hz, 1 H, H-2′), 7.93 (m, 1 H, H-6′), 7.83 (s, 1 H, H-8), 7.48 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 4.38 (t, J = 6.3 Hz, 2 H, CH_2OAr), 3.58 (t, J = 4.5 Hz, 4 H, morpholino methylene), 3.05 (s, 3 H, $S(O)CH_3$), 2.48 (partially obscured t, J = 6.3 Hz, 2 H, N$CH_2CH_2CH_2OAr$), 2.39 (br s, 4 H, morpholino methylene), 1.98 (quintet, J = 6.3 Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{21}H_{23}ClFN_5O_3S\cdot H_2O$) C, H, N.

A suspension of **71c** (153 mg, 0.319 mmol) in *i*-PrOH was reacted with NH_3(g) as above to give, after chromatography on silica gel, eluting with MeOH/CH_2Cl_2/EtOAc (3:6:10), 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]pyrido[3,2-*d*]pyrimidine (**72c**) (125 mg, 91%): mp (MeOH/CH_2Cl_2/hexane) 192−194 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.26 (s, 1 H, NH), 8.43 (s, 1 H, H-2), 8.40 (dd, J_{H-F} = 6.9 Hz, J = 2.7 Hz, 1 H, H-2′), 7.85 (m, 1 H, H-6′), 7.42 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 7.25 (s, 1 H, H-8), 6.68 (br s, 2 H, NH_2), 4.23 (t, J = 6.2 Hz, 2 H, CH_2OAr), 3.59 (t, J = 4.4 Hz, 4 H, morpholino methylene), 2.52 (partially obscured t, J = 6.2 Hz, 2 H, N$CH_2CH_2CH_2OAr$), 2.40 (br s, 4 H, morpholino methylene), 1.98 (quintet, J = 6.2 Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{20}H_{22}ClFN_6O_2\cdot0.5H_2O$) C, H, N.

A solution of **72c** (0.39 g, 0.90 mmol) in THF (40 mL) was reacted with the mixed anhydride of 2,4,6-trichlorobenzoyl chloride and acrylic acid as described above. Chromatography on silica gel, eluting with MeOH/CH_2Cl_2/EtOAc (3:6:10), gave **25** (138 mg, 31%): mp (EtOAc/Et_2O) 185−186 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.32 (s, 1 H, CONH), 9.76 (s, 1 H, NH), 8.64 (s, 1 H, H-2), 8.37 (m, 1 H, H-2′), 7.92 (m, 1 H, H-6′), 7.68 (s, 1 H, H-8), 7.44 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 6.63 (dd, J = 17.2, 10.3 Hz, 1 H, C*H*═CH_2), 6.32 (dd, J = 17.2, 1.7 Hz, 1 H, CH═CH_2), 5.83 (dd, J = 10.3, 1.7 Hz, 1 H, CH═CH_2), 4.27 (t, J = 5.9 Hz, 2 H, CH_2OAr), 3.57 (t, J = 4.6 Hz, 4 H, morpholino methylene), 2.45 (t, J = 5.9 Hz, 2 H, NCH_2CH_2-CH_2OAr), 2.37 (br s, 4 H, morpholino methylene), 1.92 (quintet, J = 5.9 Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{23}H_{24}ClFN_6O_3\cdot0.5H_2O$) C, H, N.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]pyrido[3,2-*d*]pyrimidin-6-yl]-*N*-methylacrylamide (26).** Excess aqueous methylamine (40%, 25 mL) was added to a solution of **71c** (0.50 g, 1.04 mmol) in DMSO (50 mL), and the mixture was heated in a sealed pressure vessel at 110 °C for 18 h, before being cooled, diluted with water and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the concentrate on silica gel, eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (2:3:5), gave 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-(methylamino)-7-[3-(4-morpholinyl)propoxy]-pyrido[3,2-*d*]pyrimidine (**75**) (322 mg, 69%): mp (CH_2Cl_2/Et_2O) 174−176 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (br s, 1 H, $NHAr_2$), 8.55 (s, 1 H, H-2), 8.09 (dd, J_{H-F} = 6.5 Hz, J = 2.7 Hz, 1 H, H-2′), 7.68 (m, 1 H, H-6′), 7.16 (dd, J_{H-F} = 8.8 Hz, J = 8.8 Hz, 1 H, H-5′), 7.12 (s, 1 H, H-8), 5.60 (q, J = 4.9 Hz, 1 H, N*H*CH_3), 4.23 (t, J = 6.3 Hz, 2 H, CH_2OAr), 3.74 (t, J = 4.6 Hz, 4 H, morpholino methylene), 3.18 (d, J = 4.9 Hz, 3 H, NHCH_3), 2.55 (t, J = 6.3 Hz, 2 H, N$CH_2CH_2CH_2OAr$), 2.49 (br s, 4 H, morpholino methylene), 2.08 (quintet, J = 6.3 Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{21}H_{24}ClFN_6O_2$) C, H, N.

A solution of **75** (0.235 g, 0.526 mmol) in THF (40 mL) was reacted with the mixed anhydride of 2,4,6-trichlorobenzoyl chloride and acrylic acid as described above, except that the reaction was performed at 50 °C for 48 h, with additional mixed anhydride (7.5 mol equiv) in THF (20 mL) being added after 24 h. Workup as described previously (without aqueous MeOH treatment), followed by chromatography on silica gel and elution with MeOH/CH_2Cl_2/EtOAc (3:6:10), gave **26** (164 mg, 62%): mp (EtOAc/Et_2O) 176−178 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 10.08 (s, 1 H, CONH), 8.67 (s, 1 H, H-2), 8.33 (m, 1 H, H-2′), 7.99 (m, 1 H, H-6′), 7.80 (s, 1 H, H-8), 7.45 (dd, J_{H-F} = 9.1 Hz, J = 9.1 Hz, 1 H, H-5′), 6.17 (dd, J = 16.6, 2.5 Hz, 1 H, CH═CH_2), 6.09 (dd, J = 10.0, 16.6 Hz, 1 H, C*H*═CH_2), 5.58 (dd, J = 10.0, 2.5 Hz, 1 H, CH═CH_2), 4.27 (t, J = 6.0 Hz, 2 H, CH_2OAr), 3.57 (t, J = 4.5 Hz, 4 H, morpholino methylene), 3.31 (obscured s, 3 H, NCH_3), 2.38 (partially obscured t, J =

6.0 Hz, 2 H, $NCH_2CH_2CH_2OAr$), 2.34 (br s, 4 H, morpholino methylene), 1.89 (quintet, $J = 6.0$ Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{24}H_{26}ClFN_6O_3$) C, H, N.

***N*-[4-[*N*-(3-Chloro-4-fluorophenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]pyrido[3,2-*d*]pyrimidin-6-yl]-acrylamide (27).** Mesylation of **74** (2.69 g, 6.80 mmol) as described previously, followed by treatment of the crude mesylate with 4-methylpiperazine (3.77 mL) in THF (150 mL) at reflux for 5 days, with additional 4-methylpiperazine (5.0 mL) added after day 3, gave after workup as above, 4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]-6-(methylthio)pyrido[3,2-*d*]pyrimidine (**70d**) (2.23 g, 69%): mp (EtOAc/Et_2O) 162−164 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (br s, 1 H, NH), 8.68 (s, 1 H, H-2), 8.10 (dd, $J_{H-F} = 6.5$ Hz, $J = 2.8$ Hz, 1 H, H-2′), 7.67 (m, 1 H, H-6′), 7.25 (s, 1 H, H-8), 7.19 (dd, $J_{H-F} = 8.8$ Hz, $J = 8.8$ Hz, 1 H, H-5′), 4.26 (t, $J = 6.2$ Hz, 2 H, CH_2OAr), 2.67 (s, 3 H, SCH_3), 2.61 (t, $J = 6.2$ Hz, 2 H, $NCH_2CH_2CH_2OAr$), 2.55−2.42 (br s, 8 H, piperazine methylenes), 2.30 (s, 3 H, NCH_3), 2.10 (quintet, $J = 6.2$ Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{22}H_{26}ClFN_6OS$) C, H, N.

A solution of **70d** (2.13 g, 4.47 mmol) in 1 N HCl (300 mL) was reacted with $NaIO_4$ as described above to give 4-[*N*-(3-chloro-4-fluorophenyl)amino]-6-(methanesulfinyl)-7-[3-(4-methyl-1-piperazinyl)propoxy]pyrido[3,2-*d*]pyrimidine (**71d**) (2.02 g, 92%): mp (CH_2Cl_2/Et_2O) 169−171 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.19 (s, 1 H, NH), 8.70 (s, 1 H, H-2), 8.29 (m, 1 H, H-2′), 7.93 (m, 1 H, H-6′), 7.83 (s, 1 H, H-8), 7.49 (dd, $J_{H-F} = 9.0$ Hz, $J = 9.0$ Hz, 1 H, H-5′), 4.39 (t, $J = 5.8$ Hz, 2 H, CH_2OAr), 3.06 (s, 3 H, $S(O)CH_3$), 3.00 (br s, 6 H, $NCH_2CH_2CH_2OAr$, piperazine methylene(×2)), 2.70−2.55 (br s, 7 H, NCH_3, piperazine methylene(×2)), 2.02 (br s, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{22}H_{26}ClFN_6O_2S$) C, H, N.

A suspension of **71d** (1.92 g, 3.89 mmol) in *i*-PrOH was reacted with NH_3(g) as described previously to give, after chromatography on alumina, eluting with CH_2Cl_2/EtOAc (1:1) to MeOH/CH_2Cl_2/EtOAc (1:9:10), 6-amino-4-[*N*-(3-chloro-4-fluorophenyl)amino]-7-[3-(4-methyl-1-piperazinyl)propoxy]pyrido[3,2-*d*]pyrimidine (**72d**) (1.24 g, 71%): mp (EtOAc/Et_2O) 190−191 °C; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 9.22 (s, 1 H, NH), 8.43 (d, $J = 0.7$ Hz, 1 H, H-2), 8.40 (m, 1 H, H-2′), 7.84 (m, 1 H, H-6′), 7.41 (dd, $J_{H-F} = 9.1$ Hz, $J = 9.1$ Hz, 1 H, H-5′), 7.24 (s, 1 H, H-8), 6.65 (br s, 2 H, NH_2), 4.22 (t, $J = 6.1$ Hz, 2 H, CH_2OAr), 2.51 (partially obscured t, $J = 6.1$ Hz, 2 H, $NCH_2CH_2CH_2OAr$), 2.41−2.29 (br s, 8 H, piperazine methylenes), 2.14 (s, 3 H, NCH_3), 1.96 (quintet, $J = 6.1$ Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{21}H_{25}ClFN_7O$) C, H, N.

A solution of **72d** (0.60 g, 1.35 mmol) in THF (80 mL) was reacted with the mixed anhydride of 2,4,6-trichlorobenzoyl chloride and acrylic acid as described above. Chromatography of the crude product on silica gel, eluting with MeOH/CH_2Cl_2/EtOAc (1:1:2) containing 1% Et_3N, gave **27** (223 mg, 33%): mp (EtOAc/Et_2O) 166−168 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.33 (s, 1 H, CONH), 9.79 (s, 1 H, NH), 8.64 (s, 1 H, H-2), 8.36 (dd, $J = 6.7$ Hz, $J = 2.2$ Hz, 1 H, H-2′), 7.92 (m, 1 H, H-6′), 7.67 (s, 1 H, H-8), 7.44 (dd, $J_{H-F} = 9.2$ Hz, $J = 9.2$ Hz, 1 H, H-5′), 6.63 (dd, $J = 17.1$, 10.3 Hz, 1 H, $CH{=}CH_2$), 6.32 (dd, $J = 17.1$, 2.5 Hz, 1 H, $CH{=}CH_2$), 5.84 (dd, $J = 10.3$, 2.5 Hz, 1 H, $CH{=}CH_2$), 4.26 (t, $J = 6.0$ Hz, 2 H, CH_2OAr), 2.44 (t, $J = 6.0$ Hz, 2 H, $NCH_2CH_2CH_2OAr$), 2.40−2.29 (br s, 8 H, piperazine methylenes), 2.16 (s, 3 H, NCH_3), 1.91 (quintet, $J = 6.0$ Hz, 2 H, $NCH_2CH_2CH_2OAr$). Anal. ($C_{24}H_{27}ClFN_7O_2 \cdot 0.5H_2O$) C, H, N.

Aqueous Stability Study of 18 and 25. Stock solutions of the compounds in DMSO were diluted into phosphate buffers at pH 2.6, 6.67, and 10.75. The solutions were kept at 37 °C, and HPLC traces were made at time zero and at other time points out to 24 h. The peak areas of the parent drug and the amine hydrolysis product were calculated as a percent of the $t = 0$ value. The HPLC conditions were: column, Zorbax SB-C18, 4.6 mm × 25 cm; mobile phase, 0.45 M formate buffer (ammonium formate + formic acid, pH 3.45), 80% acetonitrile, 20% MilliQ water; gradient elution, beginning aqueous/organic phase ratio 1:9, altering over 25 min to 100:0 and kept at 100:0 for another 5 min. Flow rate was 1.0 mL/min, and detection was by UV at 254 nm.

Mass Spectrometry. Solutions of compounds **18** and **25** in DMSO were added to a solution containing 25 μg of EGF receptor tyrosine kinase protein (in 20 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM EDTA) and small amounts of protease inhibitors aprotinin and leupeptin and diluted with 75 mM ammonium bicarbonate (pH 7.5). The reaction was quenched after 90 min upon addition of 5% (v/v) acetic acid, and the protein was purified and concentrated by centrifugal filtration (Microcon-10, 10-kDa cutoff filter, Amicon, Inc., Beverly, MA). The molecular weight of the protein−drug complex in a denaturing solution (80% CH_3CN, pH 2.5) was determined by ESI-MS (MAT 900Q mass spectrometer, Finnigan MAT, Bremen, Germany) equipped with a low-flow micro-ESI source operating at 150 nL/min. A portion of drug-bound protein was reduced, alkylated, and digested with trypsin. Peptide sequence analysis was performed by LC−ESI-MS/MS with a quadrupole ion trap mass spectrometer (Finnigan LCQ, Finnigan Corp., San Jose, CA) interfaced with a Michrom BioResources Magic 2002 HPLC (Auburn, CA). Peptides were eluted from the 0.3- × 15-mm Vydac C18 column directly into the mass spectrometer with a linear gradient of CH_3CN at 5 $\gamma\mu$L/min as follows: 5% solvent B to 95% solvent B over 10 min (where A = 0.05% TFA/2% CH_3CN and B = 0.045% TFA/90% CH_3CN).

Cell Culture. A431 human epidermoid 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. For growth inhibition assays, dilutions of the designated compound in 10 mL were placed in 24-well Linbro plates (1.7 × 1.6 cm, flat bottom) followed by the addition of cells (2×10^4) in 2 mL of media. The plates were incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2 in air. Cell growth was determined by cell count with a Coulter model AM electronic cell counter (Coulter Electronics, Inc., Hialeah, FL).

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 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 was then washed five 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 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 were made similar to the first set of cells.

Western Blotting Procedure. Extracts were made by lysing cells 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.

Caco-2 Cell Permeability. The absorptive and secretory transport of compounds **18** and **25** were carried out in Caco-2 cells.[24] Apical-to-basolateral (A→B) and basolateral-to-apical (B→A) experiments were performed in side-by-side diffusion apparatus with 25 μM of drug. [^{14}C]Mannitol was used to monitor cell integrity and [^{3}H]metoprolol, which is 90−95% absorbed in human,[30,31] was used as a reference compound. Cells were at passage 35 or 21, 23 or 25 days post-seeding, with an average TEER measurement of 430−508. The incubation solutions were prepared in Hank's balanced salt solution (HBSS) with 2% ethanol and 2% DMSO; pH was 6.5 and 7.4, respectively, in apical and basolateral compartments. Bidirectional transport experiments of [^{3}H]vinblastine were performed simultaneously for confirmation of P-gp activity.[25,26] Drug concentrations were monitored using an LC−MS/MS method; reference compounds were measured using scintillation counting.

The effect of **18** and **25** on P-glycoprotein transport was carried out using Caco-2 cells of passage 21, 21 days post-seeding with an average TEER measurement of 484. Apical-to-basolateral (A→B) and basolateral-to-apical (B→A) control experiments were performed in side-by-side diffusion apparatus with [^{3}H]vinblastine in the donor compartment. The compounds (25 μM) were added to both apical and basolateral compartments in B→A experiments to examine its inhibitory effects on [^{3}H]vinblastine efflux. Cyclosporin (10 μM) was also used as a positive control inhibitor,[27,28] and [^{14}C]mannitol was used to monitor cell integrity. The incubation solutions were prepared in Hank's balanced salt solution (HBSS) buffer (pH 6.5 apical, pH 7.4 basolateral) with 2% EtOH and 2% DMSO as cosolvents. [^{14}C]Mannitol permeability values indicated that the cell monolayers remained viable throughout these studies.

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 was maintained by serial passage in nude mice (NCr *nu/nu*). Nude mice were also used as tumor host for anticancer agent evaluations against this tumor model. In each experiment, 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 3. Whenever possible test compounds were evaluated over a range of dose levels ranging from toxic to ineffective.

The doses reported in Table 3 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. Derivatives **8** and **18** were administered as solutions of the isethionate salt generated in situ by the addition of 1.5 equiv of aqueous isethionic acid followed by dilution to dosing volume with distilled water (final pH 4). Compound **25** was dissolved directly in 50 mM sodium lactate buffer, pH 4. 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. Calculations of tumor growth inhibition (% T/C) and tumor growth delay (T−C) were performed as described previously.[32−35]

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