Synthesis and Structure−Activity Relationships of 6,7-Disubstituted 4-Anilinoquinoline-3-carbonitriles. The Design of an Orally Active, Irreversible Inhibitor of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor (EGFR) and the Human Epidermal Growth Factor Receptor-2 (HER-2)

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A series of of 6,7-disubstituted-4-anilinoquinoline-3-carbonitrile derivatives that function as irreversible inhibitors of EGFR and HER-2 kinases have been prepared. These inhibitors have, at the 6-position, butynamide, crotonamide, and methacrylamide Michael acceptors bearing water-solublilizing substituents. These compounds were prepared by acylation of 6-amino-4-(arylamino)quinoline-3-carbonitriles with unsaturated acid chlorides or mixed anhydrides. We performed competitive reactivity studies showing that attaching a dialkylamino group onto the end of the Michael acceptor results in compounds with greater reactivity due to intramolecular catalysis of the Michael addition. This, along with improved water-solubility results in compounds with enhanced biological properties. We present molecular modeling results consistent with the proposed mechanism of inhibition. One compound, **5** (EKB-569), which shows excellent oral in vivo activity, was selected for further studies and is currently in phase I clinical trials for the treatment of cancer.

Growth factors are important mediators of cell proliferation. The interaction of these growth factors with their respective receptors primes the signal transduction pathway. The intracellular domains of these receptor proteins function as protein tyrosine kinases, and it is this property that allows them to effect signaling. Under certain conditions, such as overexpression, mutation, or coexpression of the ligand and the receptor, these receptors can become constitutively active; the result of this is uncontrolled cell proliferation.[1] Epidermal Growth Factor Receptor (EGFR) kinase (also known as erb-B1 or HER-1) and the related Human Epidermal Growth Factor Receptor-2 HER-2 (also known as erbB-2 or *neu*) are among the growth factor receptor kinases that have been implicated as being important in cancer. EGFR is overexpressed in numerous tumors[2] including those derived from the brain, lung, bladder, head, and neck. Such overexpression has been correlated with poor prognosis in some of these diseases. The gene product is frequently mutated in the extracytoplasmic domain of the receptor in gliomas,[3] prostate cancer,[4] breast cancer,[5] and in non-small-cell lung cancer;[6] this mutation makes the receptor constitutively active.[7] In addition, the receptor need not be overexpressed or mutated to be activated, since the ligands for the receptor (EGF or Transforming Growth Factor (TGFα)) can be produced within the same cancerous tissue or cell that expresses EGF-R. This suggests that paracrine or autocrine loops stimulate hyperproliferation as in prostate cancer,[8] head and neck cancer,[9] ovarian cancer,[10] non-small-cell lung cancer,[11] and bladder cancer.[12] EGF-R deregulation has also been implicated in other diseases including polycystic kidney disease,[13] psoriasis,[14] and asthma.[15]

Since the tyrosine phosphorylation event catalyzed by EGFR or HER-2 propagates the signal for cell division and since deregulation of these kinases has been associated with disease, an inhibitor of this event may have potential therapeutic value. Since these two receptor kinases have a high sequence homology in their catalytic domains,[16] it is expected that inhibitors would function in a similar manner with each enzyme.

We previously reported[17] the discovery and EGFR kinase inhibitory activity of a class of 4-anilinoquinoline-3-carbonitriles such as **2** (see Scheme 1), which were derived, based on modeling studies, from the 4-anilinoquinazolines such as **1**.[18] In subsequent reports, we described the inhibition of Src[19] and MAP[20] kinases by members of this series. We[21] and others[22] have also described irreversible inhibitors of EGFR, such as **3**, based on the 4-anilinoquinazoline core structure that have a Michael acceptor at the 6-position. These compounds are believed to function in this manner by forming a covalent bond to a Cys residue (Cys 773 in EGFR and Cys 805 in HER-2) located in the ATP binding pocket of these enzymes. We later elaborated on this concept by introducing a water-solublizing group, as in **4**,[23] which resulted in compounds with improved biological properties. We now describe the synthesis and SAR of a series of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles, as exemplified by **5**, which represent an amalgamation of these ideas. These compounds are

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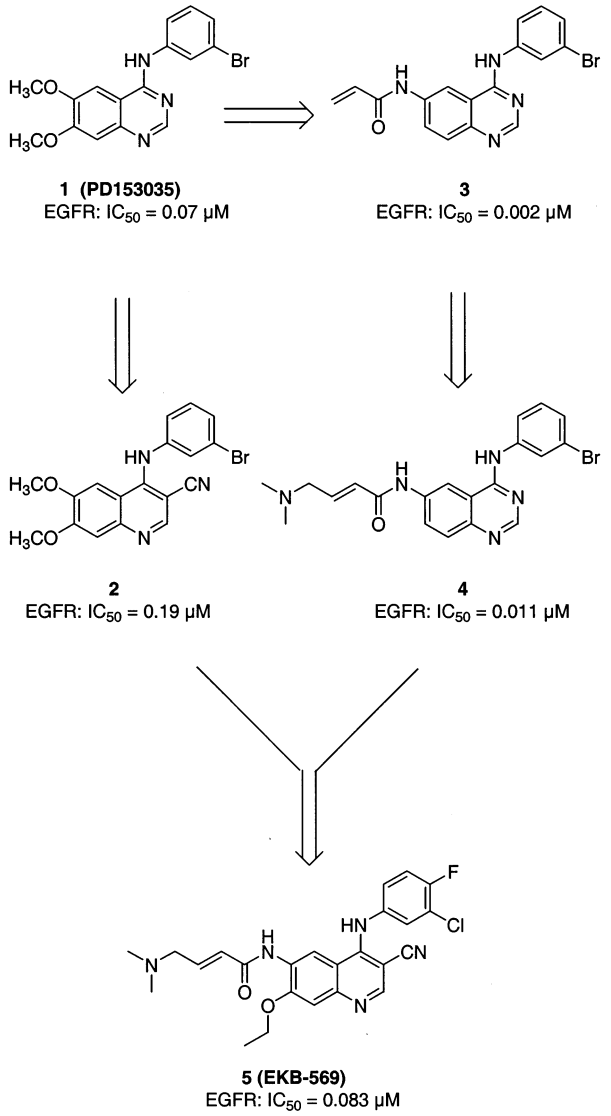
Scheme 1

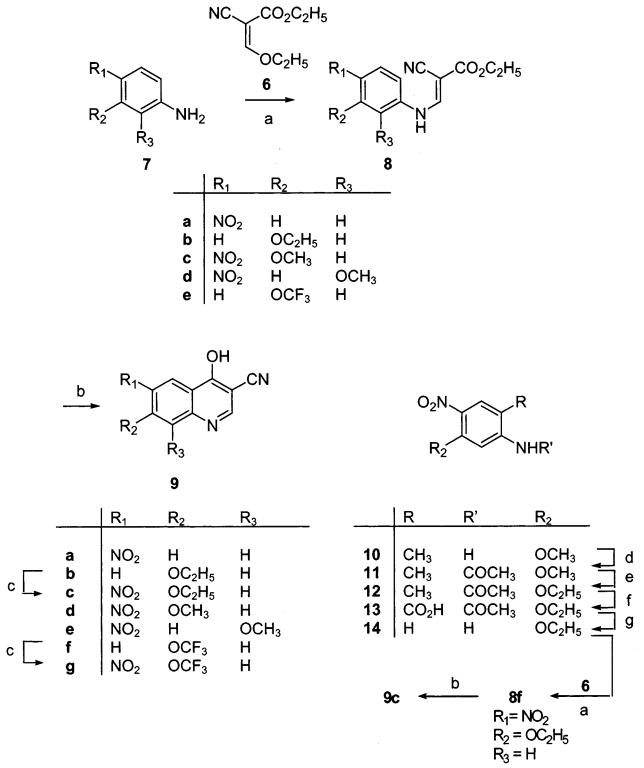


potent, irreversible inhibitors of EGFR and HER-2; one member of the series, **5** (EKB-569),[24] is currently in phase I clinical trials for the treatment of cancer.

Chemistry

In their work on the 4-anilinoquinazoline series, the Parke-Davis group adopted the strategy of attaching a water-solublizing dialkylamino group to the end of an appendage at the C-7 position while retaining an acrylamide type Michael acceptor at the C-6 position.[25] In our earlier work on the quinazoline series[23] and in our present efforts on the 3-cyanoquinoline series, we adopted a somewhat different strategy that locates the water-solublizing group directly on the Michael acceptor attached at the C-6 position. This approach, as we have shown, has the feature that when the water-solublizing group is a dialkylamino group, if placed appropriately, it can accelerate Michael additions of sulfhydryl containing species to the drug. This affords us the opportunity to modulate the reactivity of these inhibitors and thereby influence their biological activity. The syntheses of the intermediate 6-amino-4-anilinoquinoline-3-carbonitriles, **17a**−**h**, needed to prepare these compounds are shown in Schemes 2 and 3. The 4-hydroxyquinolines were prepared by the method of Bredereck.[26] Condensation of anilines **7a**−**e** with ethyl (ethoxymethylene)cyanoacetate gave the intermediates **8a**−**e** which on thermal cyclization in refluxing Dowtherm gave, regiospecifically, **9a**,**b**,**e**,**f.** The 4-hydroxyquinolines **9b** and **9f** could be nitrated selectively at the 6-position using ammonium nitrate in trifluoroacetic anhydride giving **9c** and **9g**, respectively.

Scheme 2[a]

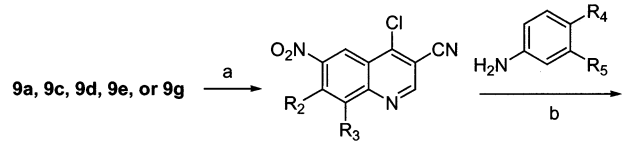


[a] (a) Toluene, reflux. (b) Dowtherm, 258 °C. (c) NH_4NO_3, TFA anhydride. (d) $(CH_3CO)_2O$, CH_3CO_2H. (e) i. LiCl, DMF, reflux; ii. C_2H_5I, K_2CO_3, reflux. (f) $KMnO_4$, $MgSO_4$, H_2O, reflux. (g) H_2SO_4, H_2O, reflux.

An alternate synthesis for **9c** was developed which avoided the nitration step, which could have been problematic in large-scale work. The amino group of **10** was protected as its acetate. The methoxy group of **11** was converted to the ethoxy group of **12** using a two step, single-pot reaction, involving refluxing **11** in DMF containing an excess of lithium chloride. After the gaseous methyl chloride evolved, refluxing was continued in the presence of ethyl iodide and potassium carbonate. The methyl group of **12** was removed by permanganate oxidation to the carboxylic acid **13** followed by a combined acetate hydrolysis and decarboxylation in refluxing aqueous sulfuric acid. The resulting aniline **14** was then converted to **9c** using the method described above. The 4-hydroxyquinolines **9a**,**c**−**e**,**g** on treatment with refluxing $POCl_3$ gave the 4-chloroquinoline-3-carbonitriles **15a**−**e**.

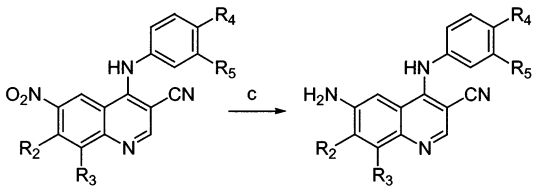
In our earlier work in the quinazoline[23] and cyanoquinoline[18] series we observed the best activity when the aniline ring at the 4-position contained either a 3-bromo or 3-chloro-4-fluoro substitution pattern. Accordingly, we used these anilines exclusively in the present work. Refluxing **15a**−**e** with either aniline in 2-propanol gave the intermediates **16a**−**h** in good yields as the hydrochloride salts. The nitro groups in **16a**−**h**

Scheme 3[a]



15a-e

	R_2	R_3
a	H	H
b	OC_2H_5	H
c	OCH_3	H
d	H	OCH_3
e	OCF_3	H



16a-h **17a-h**

	R_2	R_3	R_4	R_5
a	H	H	H	Br
b	OC_2H_5	H	H	Br
c	OCH_3	H	H	Br
d	H	H	F	Cl
e	OC_2H_5	H	F	Cl
f	OCH_3	H	F	Cl
g	H	OCH_3	H	Br
h	OCF_3	H	F	Cl

[a] (a) $POCl_3$, reflux. (b) $(CH_3)_2CHCH$, reflux. (c) Fe, NH_4Cl, CH_3OH, H_2O, reflux.

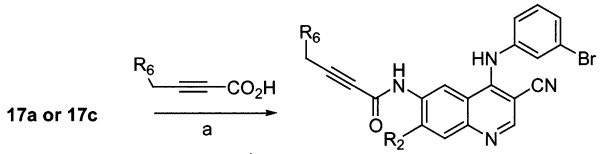
were reduced in aqueous methanol using ammonium chloride and iron powder to give the anilines **17a**−**h**.

The compounds containing the 2-butynamide type Michael acceptors **18**−**28** (Scheme 4) were prepared by the reaction of **17a** or **17c** with various 4-substituted butynoic acids using a mixed anhydride method and *N*-methylmorpholine as a base catalyst. The preparations of the 4-substituted butynoic acids are described in our earlier work.[23]

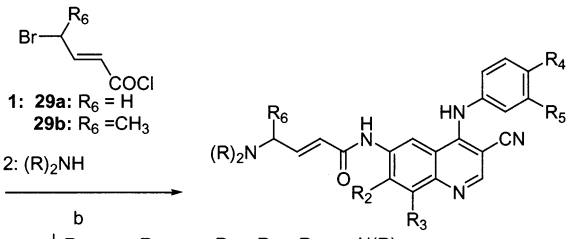
The compounds in the crotonamide series **5** and **30**−**45** were prepared as shown in Scheme 4. Condensation of **17a**−**h** with the acid chlorides **29a** or **29b** gave intermediate 4-bromo-2-alkenoic acid amides. These bromo derivatives were contaminated (10−20%) with the corresponding γ-chloro compounds. Nucleophilic displacement with a secondary amine yielded 4-substituted crotonamides **5** and **30**−**45**. In this reaction the chloride reacts slower than the bromide, but nevertheless, the reaction can be driven to completion. Alternatively, the amine product of the reaction can be separated from any unreacted chloro derivative by chromatography. The acid chlorides **29a**,**b** were prepared from the corresponding silyl esters[27] using oxalyl chloride.

Alternatively, compounds **5** and **47**−**52** were prepared (Scheme 5) by acylation with the carboxylic acid chlorides **46a**−**e** in acetonitrile or THF. The carboxylic acid chlorides **46c** and **46d**, needed to prepare compounds **51** and **52** in the methacrylamide series, were prepared from the corresponding amino acid hydrochlorides which, in turn, were prepared using a modification of the method of Krawczyk.[28] The amino acid chloride hydrochloride **46e**, which provided an alternate route to **5**, was prepared in a similar manner.

Scheme 4[a]



	R_2	R_6
18	H	H
19	H	OCH_3
20	H	$O(CH_2)_2OCH_3$
21	H	OCH_2OCH_3
22	H	thiomorpholinyl
23	H	(2S)-2-(methoxymethyl)-1-pyrrolidinyl
24	H	$N(n\text{-}Pr)_2$
25	H	$N(i\text{-}Pr)_2$
26	OCH_3	H
27	OCH_3	OCH_3
28	OCH_3	$N(CH_3)_2$



	R_2	R_3	R_4	R_5	R_6	N(R)$_2$
30	H	H	H	Br	H	$N(CH_3)_2$
31	OCH_3	H	H	Br	H	$N(CH_3)_2$
32	OCH_3	H	H	Br	H	*N*-morpholinyl
33	OC_2H_5	H	H	Br	H	$N(CH_3)_2$
34	OC_2H_5	H	H	Br	H	*N*-morpholinyl
35	H	OCH_3	H	Br	H	$N(CH_3)_2$
36	H	OCH_3	H	Br	H	*N*-morpholinyl
37	H	H	F	Cl	H	$N(CH_3)_2$
38	H	H	F	Cl	H	*N*-morpholinyl
39	OCH_3	H	F	Cl	H	$N(CH_3)_2$
40	OCH_3	H	F	Cl	H	$N(C_2H_5)_2$
41	OCH_3	H	F	Cl	H	*N*-morpholinyl
42	OCF_3	H	F	Cl	H	$N(CH_3)_2$
43	OCH_3	H	F	Cl	CH_3	$N(CH_3)_2$
44	OCH_3	H	F	Cl	CH_3	$N(C_2H_5)_2$
45	OCH_3	H	F	Cl	CH_3	*N*-morpholinyl
5	OC_2H_5	H	F	Cl	H	$N(CH_3)_2$

[a] (a) *i*-BuOCOCl, *N*-methylmorpholine, THF, *N*,*N*-diisopropylethylamine, 0−25 °C. (b) THF, 0 °C.

For the reactivity studies described below, we needed a sample of the quaternary ammonium derivative **53**. This could simply be prepared by the reaction of **5** with methyl iodide in THF.

Molecular Modeling

A homology model for the catalytic domain of EGFR kinase was described in detail in a previous paper.[17] It was constructed using a combination of two closely related crystal structures as templates, FGF Receptor-1 for the N-terminal lobe and Hematopoietic Cell Kinase (HcK) for the C-terminal lobe. The inhibitor **5** was docked into the ATP binding site of this model of EGFR kinase with an orientation similar to that used in our previous work. A 7.5 Å sphere of water molecules was added around the ligand, and a short (3ps) dynamics run was carried out, followed by several cycles of minimization using Quanta/CHARMm. The entire protein−ligand−water complex was allowed to move during these calculations.

Scheme 5[a]

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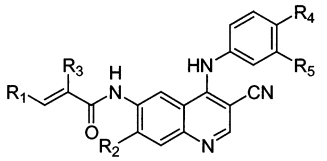
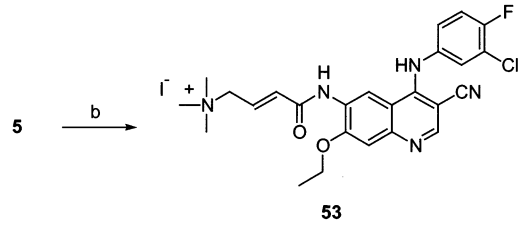
	R_1	R_3
a	H	H
b	CH_3	H
c	H	CH_2-N-morpholinyl
d	H	$CH_2N(CH_3)_2$
e	$CH_2N(CH_3)_2$	H



	R_1	R_2	R_3	R_4	R_5
47	H	H	H	H	Br
48	H	OCH_3	H	H	Br
49	CH_3	H	H	H	Br
50	CH_3	OC_2H_5	H	F	Cl
51	H	H	CH_2-*N*-morpholinyl	F	Cl
52	H	OC_2H_5	$CH_2N(CH_3)_2$	F	Cl
5	$CH_2N(CH_3)_2$	OC_2H_5	H	F	Cl



53

[a] (a) THF or CH_3CN, Hunig's base or *N*-methylmorpholine, 0 °C. (b) CH_3I, THF.

In the final model of the complex with **5** (Figure 1), the N1 atom of the cyanoquinoline is hydrogen-bonded to the backbone NH of Met 769, and the nitrogen atom of the 3-cyano group interacts with the hydroxyl group of Thr 830. In our earlier work with the quinazoline series of inhibitors, this threonine residue forms a hydrogen bond to a water molecule. This water molecule, in turn, forms a hydrogen bond to the N3 atom of the quinazoline. The C2 atom of the cyanoquinoline is 4.4 Å from the backbone carbonyl oxygen of Gln 767, and the C8 atom is 3.01 Å from the backbone carbonyl oxygen of Met 769. The 4-fluoro-3-chloroaniline moiety lies in a hydrophobic pocket containing Val 702, Ala 719, and Thr 766. Most significantly, the β-carbon atom of the Michael acceptor side chain of **5** is located 4.3 Å from the sulfhydryl group of Cys 773 and should be easily accessible for covalent interaction. Additionally, the N-atom of the dimethylamino group on **5** is located 3.82 Å away from the sulfhydryl hydrogen of Cys 773. We have proposed earlier for the quinazoline series,[23] and are now proposing for these related 3-cyanoquinolines, that this dimethylamino group can serve as an intramolecular base catalyst for Michael additions to this inhibitor. Also, given the arrangement of the functionalities predicted by this binding model, we are proposing that this intramolecular catalysis operates after **5** binds at the active site of EGFR (or HER2) and that the Michael addition reaction to bound **5** will be accelerated due to the entropic effect of having the reactive center, nucleophile, and base catalyst in close proximity. Finally, this binding model indicates that the water-solubilizing dimethylamino group of **5** points out of the ATP binding pocket toward the solvent environment justifying the decision to place it at the end of the Michael acceptor.

Since EGFR and HER-2 kinases have a high sequence homology in their catalytic domains,[16] and since Cys 773 is conserved in HER-2 (numbered Cys 805), we are assuming that our inhibitors will be binding in a similar manner to the latter enzyme.

Reactivity Studies

In an earlier report we showed that compound **5** functions as an irreversible inhibitor of EGFR.[24] We demonstrated this by incubating radiolabeled **5** with a membrane preparation derived from A431 cells that overexpress EGFR. We found that a single protein incorporated labeled drug and that this protein had a mass corresponding to EGFR. In addition, this protein could be immunoprecipitated with an antibody specific for EGFR. Since we are proposing that these inhibitors function by forming a covalent linkage to Cys 773 of EGFR (and Cys 805 of HER-2) and since the reactivity of these inhibitors might have some bearing on their biological activity, we performed competitive reactivity studies with these inhibitors using the tripeptide glutathione as a surrogate for the enzymes. In our previous work on the quinazoline series we showed that a 4-dialkylaminobut-2-enamide type of Michael acceptor as in **4** is significantly more reactive than a simple but-2-enamide Michael acceptor lacking a terminal dialkylamino group. This difference in reactivity was demonstrated in a competition experiment using a limited amount of glutathione in an aqueous/organic solvent mixture. We now report the results of a more limited reactivity study within the 4-anilinoquinoline-3-carbonitrile series. This additional study was designed to address questions not covered in our earlier work and to address the origin of the increased reactivity of the 4-dialkylaminobut-2-enamide moiety. These data are summarized in Table 1. In each reaction solution, consisting of a THF−H_2O−CH_3OH mixture, two inhibitors (2.5 mM each) were allowed to compete with a limiting quantity of glutathione (1.25 mM). Each reaction was carried out at ambient temperature for the time stated in the table. The glutathione conjugates were detected by LC/MS, and the area percents of the conjugate and unreacted inhibitor were measured. The reaction conditions were adjusted to give low conversion to glutathione conjugates to ensure that the ratios reflect kinetic control.

Consider the two inhibitors **5** and **50** that differ only by the presence of a dimethylamino group in **5**. While we observed a Michael addition reaction of glutathione to **5** (19% conversion), we detected no addition to **50** within the limits of detection of our analytical method. Evidently **5** is much more reactive than **50**. In our earlier work with the quinazoline series,[23] we proposed two possible explanations for this enhanced reactivity. First, under the aqueous reaction conditions (or under physiological conditions) it is expected that the dialkylamino group of inhibitors such as **5** would be protonated to a significant extent and that the resulting positive charge could lead to an increase in the electrophilicity

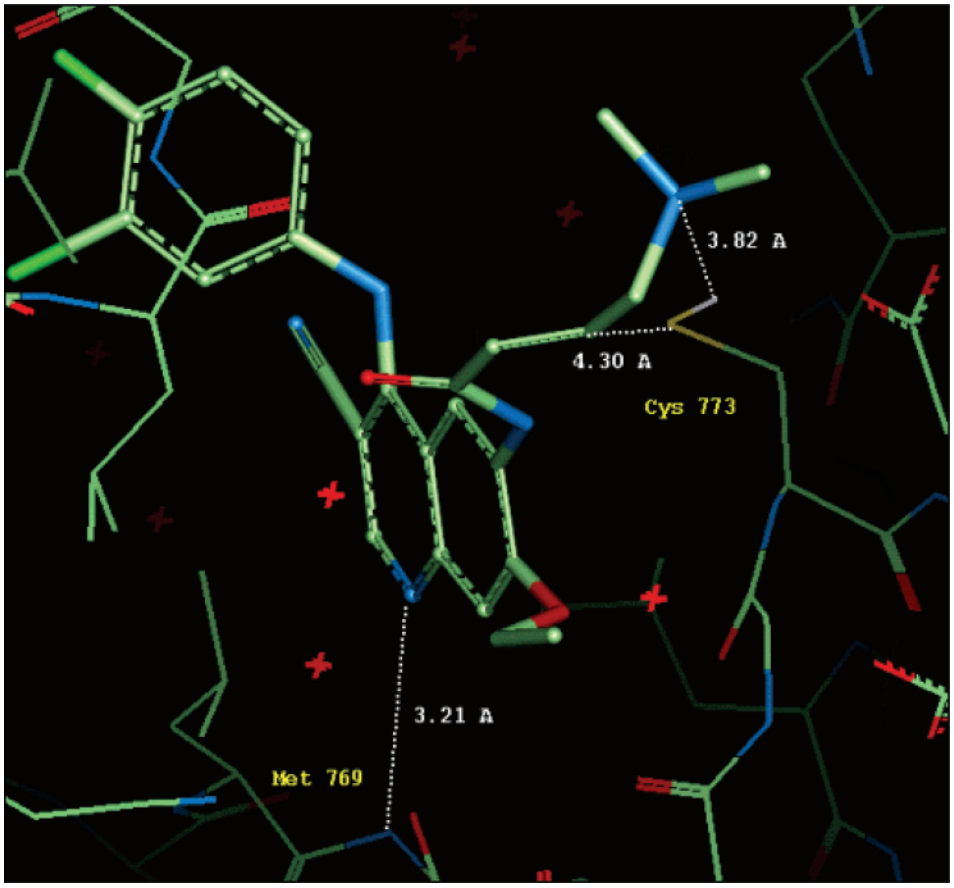
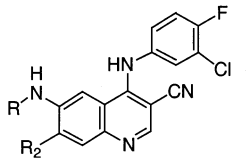
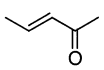
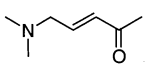
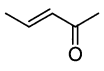
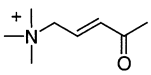
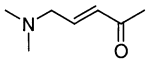
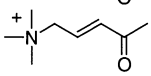
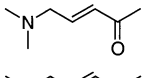
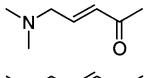
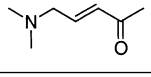
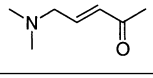


Figure 1. Proposed binding model for 3-cyanoquinoline **5** built using the homology model for EGFR kinase. Key distances are shown in the figure and discussed in detail in the text.

Table 1. Competitive Reaction of Selected 4-Anilinoquinoline-3-carbonitriles with Glutathione

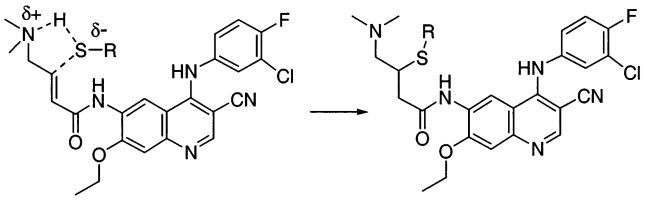


Compound A			Compound B			Time[a] (hr)	Glutathione Adduct[b] % conversion	
	R	R_2		R	R_2		A-glutathione	B-glutathione
50		OC_2H_5	**5**		OC_2H_5	20	0	19
50		OC_2H_5	**53**		OC_2H_5	21	0	0
5		OC_2H_5	**53**		OC_2H_5	21	15	0
5		OC_2H_5	**37**		H	22	13	12
5		OC_2H_5	**39**		OCH_3	22	14	14

[a] Reaction time at room temperature. [b] Calculated based on the integration of peak areas on the HPLC chromatogram. Each glutathione conjugate showed the expected mass in the mass spectrum.

of the double bond through an inductive effect. Second, we proposed that the dialkylamino group at the end of the Michael acceptor could serve as an intramolecular base catalyst by extracting the proton from the nucleophilic sulfhydryl group through a cyclic five-membered ring mechanism as shown in Scheme 6. Note that the diagram in this scheme is not intended to imply that the proton abstraction by the dimethylamino group and the addition of the sulfur nucleophile to the double bond is a concerted process. The mechanism might very well

Scheme 6



involve a discrete ion pair intermediate that then collapses to product. For compounds in the methacrylamide series, such as **51** and **52**, we propose a similar cyclic six-membered ring mechanism.

To determine if the enhanced reactivity of the 4-dialkylaminobut-2-enamide type Michael acceptor is due to an inductive effect or to intramolecular base catalysis, we performed a competitive experiment between **5** and the quaternary ammonium derivative **53**. After 21 h, within the limits of detection of our method, we found a 15% conversion to the Michael adduct of **5** and no conversion to the Michael adduct of **53**. One would expect, as a result of the full positive charge, that an inductive effect, if operable, would be maximized in **53** compared to **5**. The result of this experiment suggests that the intramolecular base catalysis is a more important factor than the inductive effect in leading to enhanced reactivity of those inhibitors containing Michael acceptors with terminal dialkylamino groups compared to those which lack these groups. That the presence of a dialkylamino group is a necessary feature in order to see a Michael addition under these reaction conditions is shown by the competitive experiment between **50** and **53** where no adduct was observed for either compound. In addition, in our work with the quinazolines series,[23] we showed that a 4-dialkylaminobut-2-enamide Michael acceptor is significantly more reactive than a 4-alkoxybut-2-enamide indicating it is not simply the electronegativity but the basicity of the heteroatom that is the determining factor with respect to reactivity. Still, while the results of the above experiments support the intramolecular catalysis argument, these results are, perhaps, not definitive since the difference in the reactivity between **5** and **53** could be influenced by the differing steric volumes of the dimethylamino and trimethylammonium groups in these compounds, respectively.

Given the arrangement of the functional groups predicted by our binding model for compounds such as **5**, we propose that this intramolecular catalysis also occurs when the compound binds at the active site in EGFR or HER-2 and this, in part, accounts for the enhanced biological activities exhibited by such compounds.

One other issue addressed by these competition experiments concerns the influence of the 7-substituents on the reactivity of the compounds. It appears that compounds **5**, **37**, and **39** with H, OCH_3, and OC_2H_5 groups at this position, respectively, show essentially the same reactivity toward glutathione in these experiments.

Results and Discussion

Enzyme and Cellular Inhibitory Activities. The compounds shown in Table 2 were evaluated for their ability to inhibit the autophosphorylation of EGFR and HER-2 kinases using a solid-phase ELISA assay. We previously pointed out that the IC_{50} values that we measure are routinely higher than determinations made by other workers. We attribute this to differences in the nature of the enzymes and substrates that we use as well as to the overall assay conditions. We use a solid-phase ELISA-based assay. Our enzymes consist of the purified cytoplasmic domain of EGFR or HER-2, and we measure the inhibition of autophosphorylation of these proteins. Other researchers have measured inhibition using, in soluble formats, the entire enzymes purified from cells along with exogenous peptide substrates.

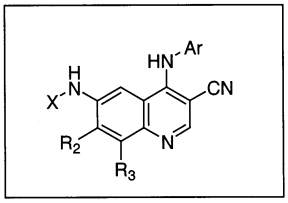
As we already found for compound **5**, a number of these inhibitors bind irreversibly to EGFR (and, presumably, HER-2). This raises some concerns about the meaning of the IC_{50} determinations since the IC_{50} value should depend on the extent to which the covalent interaction has occurred. In addition, one could argue that the IC_{50} value for such inhibitors should consist of two components, one reflecting reversible binding and another reflecting the subsequent covalent binding. One would therefore expect that the IC_{50} values would be time dependent insofar as an inhibitor that reacts more slowly with the enzyme, or does not react at all, would be expected to have a higher IC_{50} value. However, an absolute dependence of the IC_{50} value with reactivity of the inhibitors cannot be counted on due to the component of the IC_{50} that reflects reversible (noncovalent) binding.

The compounds were also evaluated for their ability to inhibit the growth of several cell lines (Table 2). Three human carcinoma cell lines were used: A431 (epidermoid) which overexpresses EGFR, SKBR3 (breast) which overexpresses HER-2 and, to a lesser extent, EGFR, and SW620 (colon) which is believed not to express either EGFR or HER-2 to a significant extent.

To get a global view of these data, a matrix of correlation coefficients was constructed and is shown in Table 3. It is evident that there is a reasonable correlation between the EGFR and HER-2 inhibitory activities. This is not surprising in view of the high sequence homology of the catalytic domains of these two kinases. In addition, we find a good correlation between the activities of the compounds in inhibiting the growth of the A431 and SKBR3 cell lines. Again, this is expected given that both these cell lines are known to express, to a large degree, and to depend on the target growth factor receptors. The correlation between the A431 and SKBR3 cell line data is significantly better than the correlation between either of these data with the SW620 cell line results.

We do not see a correlation between the enzyme activities and the ability of these compounds to inhibit the growth of the A431 and SKBR3 cell lines. Since factors other than the ability of a compound to inhibit an enzyme, such as the degree of cell penetration, will play a role here, this might be expected. Surprisingly, the enzyme data correlates better with the SW620 cell activities. What is not evident, however, just from these correlation coefficients, is that for the most part, the compounds are more potent in inhibiting the growth of the A431 and SKBR3 cell lines than the SW620 cell line.

Table 2. Inhibition of EGFR and HER-2 Kinases and Inhibition of Cell Proliferation



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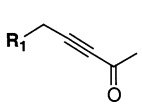
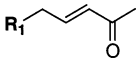


I



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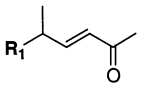

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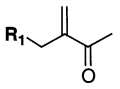
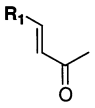


IV



V

compd	series	R_1	R_2	R_3	Ar	EGFR IC_{50}[a]	HER-2 IC_{50}[b]	A431 IC_{50}[c]	SKBR3 IC_{50}[c]	SW620 IC_{50}[c]
5	II	$N(CH_3)_2$	OC_2H_5	H	3-Cl, 4-F	0.08	1.23	0.08	0.01	0.68
18	I	H	H	H	3-Br	0.94	3.87	5.03	5.50	12.34
19	I	OCH_3	H	H	3-Br	0.96	0.30	0.28	0.35	0.52
20	I	$O(CH_2)_2OCH_3$	H	H	3-Br	0.93	0.45	0.27	0.39	0.34
21	I	OCH_2OCH_3	H	H	3-Br	0.33	0.38	0.17	0.23	0.23
22	I	thiomorpholinyl	H	H	3-Br	0.55	1.04	0.10	0.07	0.35
23	I	2(*S*)-2-(methoxymethyl-1-pyrrolidinyl	H	H	3-Br	0.15	1.22	1.20	0.54	4.14
24	I	N(*n*-Pr)$_2$	H	H	3-Br	0.13	0.37	0.24	0.08	0.40
25	I	N(*i*-Pr)$_2$	H	H	3-Br	0.25	0.32	0.14	0.04	0.25
26	I	H	OCH_3	H	3-Br	0.91	2.12	5.12	8.36	7.58
27	I	OCH_3	OCH_3	H	3-Br	0.79	0.38	0.73	0.29	1.77
28	I	$N(CH_3)_2$	OCH_3	H	3-Br	0.09	0.18	0.11	0.12	0.30
30	II	$N(CH_3)_2$	H	H	3-Br	0.31	4.16	0.45	0.18	5.48
31	II	$N(CH_3)_2$	OCH_3	H	3-Br	0.79	5.66	0.14	0.03	1.17
32	II	*N*-morpholinyl	OCH_3	H	3-Br	3.83	34.13	0.16	0.12	5.38
33	II	$N(CH_3)_2$	OC_2H_5	H	3-Br	1.60	2.97	0.13	0.03	1.15
34	II	*N*-morpholinyl	OC_2H_5	H	3-Br	10.32	33.95	0.14	0.07	2.88
35	II	$N(CH_3)_2$	H	OCH_3	3-Br	2.88	9.35	2.52	2.52	8.97
36	II	*N*-morpholinyl	H	OCH_3	3-Br	5.39	23.59	2.22	2.66	9.57
37	II	$N(CH_3)_2$	H	H	3-Cl, 4-F	0.65	1.95	0.15	0.05	0.88
38	II	*N*-morpholinyl	H	H	3-Cl, 4-F	2.29	8.74	0.41	0.22	6.33
39	II	$N(CH_3)_2$	OCH_3	H	3-Cl, 4-F	2.32	4.49	0.06	0.04	0.91
40	II	$N(C_2H_5)_2$	OCH_3	H	3-Cl, 4-F	1.91	7.80	0.07	0.05	0.66
41	II	*N*-morpholinyl	OCH_3	H	3-Cl, 4-F	13.57	31.43	0.12	0.11	6.67
42	II	$N(CH_3)_2$	OCF_3	H	3-Cl, 4-F	2.64	4.13	0.47	0.30	1.46
43	III	$N(CH_3)_2$	OCH_3	H	3-Cl, 4-F	5.22	11.82	0.43	0.19	1.13
44	III	$N(C_2H_5)_2$	OCH_3	H	3-Cl, 4-F	4.84	9.58	0.38	0.31	0.88
45	III	*N*-morpholinyl	OCH_3	H	3-Cl, 4-F	10.59	33.95	0.59	0.20	4.94
47	V	H	H	H	3-Br	0.59	1.63	0.36	0.44	3.38
48	V	H	OCH_3	H	3-Br	0.81	4.68	0.31	0.12	1.88
49	V	CH_3	H	H	3-Br	1.62	4.50	4.40	10.12	12.28
50	V	CH_3	OC_2H_5	H	3-Cl, 4-F	7.50	21.51	1.25	0.17	7.46
51	IV	*N*-morpholinyl	H	H	3-Cl, 4-F	2.70	4.39	0.26	0.22	0.49
52	IV	$N(CH_3)_2$	OC_2H_5	H	3-Cl, 4-F	0.56	1.21	0.18	0.09	0.32

[a] Concentration in μM needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50% as determined from the dose−response cure. Determinations were done in duplicate or triplicate and individual determinations differed, on average, by 77% from the mean. [b] Concentration in μM needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50% as determined from the dose−response curve. Determinations were done in duplicate and individual determinations differed, on average, by 31% from the mean. [c] Dose−response curves were determined at five concentrations. The IC_{50} values are the concentrations in μM needed to inhibit cell growth by 50% as determined from these curves. Reported IC_{50} values are averages of at least two determinations.

Table 3. Matrix of Correlation Coefficients for the Data in Table 2[a]

	EGFR (log IC_{50})	HER2 (log IC_{50})	A431 (log IC_{50})	SKBR3 (log IC_{50})	SW620 (log IC_{50})
EGFR (log IC_{50})	1.000				
HER2 (log IC_{50})	0.838	1.000			
A431 (log IC_{50})	0.171	0.178	1.000		
SKBR3 (log IC_{50})	0.186	0.101	0.914	1.000	
SW620 (log IC_{50})	0.474	0.658	0.680	0.603	1.000

[a] The Pearson product moment correlation coefficients are are calculated using the equation: $R = n(\sum XY) - (\sum X)(\sum Y)/[\{n\sum X^2 - (\sum X)^2\}\{n\sum Y^2 - (\sum Y)^2\}]^{1/2}$.

On average, the compounds are 8 times more potent in inhibiting the A431 line and 16 times more potent in inhibiting the SKBR3 line compared to the SW620 line. This is consistent with the mechanism of growth inhibition relying, in part, on the targeting of EGFR or HER-2 kinases. The fact that the SW620 line is inhibited by these compounds could suggest that the SW620 line may have some dependence on EGFR or HER-2 despite that fact that these receptors are not expressed to a large degree in this line or that these compounds have an undefined additional mechanism for inhibiting cell growth at higher concentrations.

As mentioned, we chose to prepare the inhibitors with the 3-bromo or 3-chloro-4-fluoro substitution pattern on the aniline ring since previous work suggested that this substitution pattern is consistent with optimal activity. In the current work, there is no clear preference for either substitution pattern. With respect to the compounds with simple Michael acceptors, there does not appear to be much difference between the acetylene and olefin types (compare **18** and **49**). Adding a neutral substituent to the end of the double bond decreases activity somewhat in both the enzyme and cell assays

(compare **47** and **49**). A significant enhancement in activities is observed when the substituent at the end of the Michael acceptor is a dimethylamino group. For example, **5** is a more potent inhibitor of both enzymes and the target cell lines than the related compound **50** that lacks the dimethylamino group. This result parallels the results we obtained in our reactivity studies and is consistent with our previous observations with the quinazoline-based inhibitors that there is a relationship between the reactivity of such irreversible inhibitors and their biological activities. A similar, but less pronounced, enhancement is observed for the pair of compounds **49** and **30**. There is a clear preference for compounds that contain a dimethylamino moiety as the basic group compared to the related compounds with a morpholinyl group (compare the pairs of compounds **31** and **32**, **33** and **34**, **35** and **36**, or **37** and **38**); this preference is more evident in the enzyme assays than in the cell assays. There does not seem to be much of a difference in activity between compounds with dimethylamino or diethylamino groups (compare **39** and **40**).

With respect to the substituent in the 7-position, there are only slight differences in activity between pairs of related compounds when the substituents are methoxy, ethoxy, or trifluoromethoxy. One exception is inhibitor **5** containing a 7-ethoxy group which is significantly more potent, in the enzyme assays, than the corresponding compound **39** containing a 7-methoxy group but this difference in activity is less evident in the cell assays. In other work, not shown here, we found that a number of the inhibitors that have a 7-methoxy substituent showed a very weak positive response in an Ames test after microsomal incubation while the corresponding ethoxy derivatives did not. This is a potential advantage for the 7-ethoxy derivatives compared to their 7-methoxy counterparts. Compounds with an 8-methoxy group such as **35** and **36** are clearly inferior to the corresponding compounds **31** and **32**, respectively, having a 7-methoxy group; this is evident in both the enzyme and cell assays. Adding an additional methyl group to the end of the Michael acceptor as in **43**, **44**, and **45** is slightly detrimental to the enzyme and cell activities when compared to **39**, **40**, and **41**, respectively. Finally, there is no major difference in activities between compounds in the crotonamide series such as **5** and **38** compared to the respective compounds **52** and **51** in the methacrylamide series. From these in vitro studies, it is clear that one of the best inhibitors of EGFR is **5**. However, the enzyme data suggests it is a somewhat less effective inhibitor of HER-2. With respect to the selectivity of the kinase activity of **5**, the compound was tested in src, cdk4, c-met, akt, raf, and coupled mek/erk assays. In each case, the IC_{50} value for inhibition was 1−3 orders of magnitude higher for these enzymes compared to EGFR.

In Vivo Efficacy. Compound **5** was evaluated in a nude mouse xenograft model bearing A431 human tumors that overexpress EGFR. The result is shown in Figure 2. The inhibitor was administered, at the indicated doses, orally every day for the first 10 days of the experiment. After day 10, dosing was discontinued. A clear dose−response relationship was observed. At the higher doses, the drug-treated groups showed significantly smaller tumors compared to that of the control group. At the highest dose investigated, 80 mg/kg, the compound inhibits tumor growth by over 90% even 20 days after terminating dosing. Since the compound is expected to function as a cytostatic agent, once treatment of the drug is stopped, tumor growth should return. However, in comparison with the control group, the tumor growth was greatly retarded after dosing was discontinued. It is clear that **5** shows very good antitumor activity in this model. In addition to the A431 model, **5** has been evaluated in a number of other tumor models and has also shown efficacy in these models; these data will be reported separately.

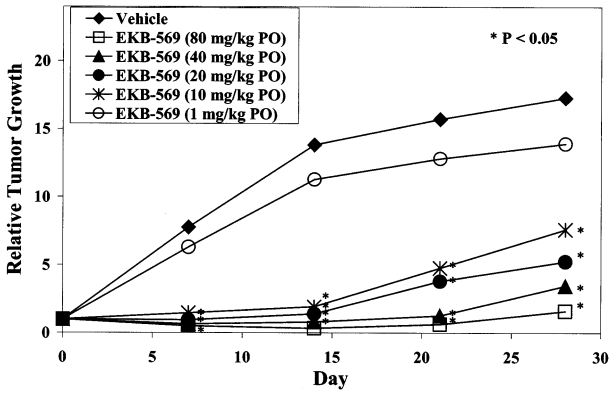


Figure 2. The effect of **5** (EKB-569) on the growth of the human epidermoid carcinoma A431 cell line in nu/nu mice. Groups of 5 female mice were injected with 5×10^6 A431 cells. Animals bearing staged tumors were treated PO for 10 consecutive days with vehicle or **5** at the doses indicated. Relative tumor growth was determined every 7 days for a period of 28 days. Data are mean fold increase in tumor volume in each group. *$P \leq 0.05$ by Student's *t*-test.

On the basis of the work presented here and after an extensive toxicological and pharmokinetic evaluation, compound **5** has been chosen as a clinical lead. It is presently in phase I clinical trial, as EKB-569, for the treatment of EGFR dependent cancers.

Conclusions

We described the synthesis and SAR of a series of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. These compounds are effective irreversible inhibitors of EGFR and HER-2 kinases. These compounds represent the culmination of our earlier efforts in designing inhibitors of these enzymes based on the 4-anilinoquinoline-3-carbonitrile and 4-anilinoquinazoline core structures. One member of this series, **5** (EKB-569), was selected for additional studies in man in order to determine its potential as a therapeutic agent for the treatment of cancer.

Experimental Section

Molecular Modeling. Homology modeling was carried out using the MOE (Molecular Operating Environment) software (Chemical Computing Group Inc. 1255 University St. Suite 1600 Montreal, Quebec, Canada H3B 3X). Initial crude minimization was performed within the homology modeling function of MOE. However, the resulting model needed further energy minimization. This was done using Quanta/CHARMm (Molecular Simulations Inc. 9685 Scranton Road, San Diego CA 92121). The crude model from MOE was minimized with a few thousand cycles of minimization using the ABNR (adopted-basis Newton−Raphson) method.

Ligands were modeled by positioning them in the active site in accordance with the published crystal structures of quinazo-

line derivatives bound to CDK2 and MAP kinase (p38)[29] and our previous modeling studies. A 7.5 Å sphere of water molecules was added around the ligand. The entire complex was then subjected to alternate cycles of minimization and dynamics. Each dynamics run was short, about 3ps. The intent was to get a satisfactory structure for the complex that was consistent with the published crystal structure.

Biology. The preparation of the enzymes, the details of our kinase autophosphorylation assays, the cell proliferation assays, and the in vivo studies are described in detail in our earlier publication.[23]

Chemistry. ^{1}H NMR spectra were determined with a NT-300 WB spectrometer at 300 MHz. Chemical shifts (δ) are expressed in parts per million relative to the internal standard tetramethylsilane. Electrospray mass spectra were recorded in positive mode on a Micromass Platform spectrometer. Electron impact and high-resolution mass spectra were obtained on a Finnigan MAT-90 spectrometer. Some high-resolution electrospray mass spectra with higher precision were obtained on a Brucker 9.4T FTMS spectrometer. Chromatographic purifications were by flash chromatography using Baker 40 μm silica gel. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected.

Competitive Reaction with Glutathione. Sample solutions were prepared by dissolving the two compounds (0.0083 mmol of each) in 1.0 mL of THF/CH_3OH (1:2) solution. A glutathione solution was prepared by dissolving 18 mg (0.0586 mmol) of glutathione in 0.5 mL of water. A 300 μL aliquot of sample solution (2.5 μmol of each compound) was dispensed into a 2 mL LC analysis vial, diluted with 689 μL of THF/CH_3OH/H_2O (1:2:1), followed by addition of 11 μL (1.25 μmol) of freshly prepared glutathione solution. A 5 μL aliquot of the reaction solution was autoinjected into a HP 1100 LC/MS every hour for 24 h. The LC column used was LUNA: ODS3 (4.6 × 150 mm, 5 μm), and the mobile phase was a gradient of 10−40% aqueous formic acid (0.05%) in acetonitrile in 12 min at a flow rate of 1 mL/min. Both a photodiode array detector and a triple quad electrospray mass spectrometer were used for detection.

5-Methoxy-2-methyl-4-nitroacetanilide (11). A solution of 182.1 g (1.0 mol) of **10** in 400 mL of acetic acid was heated to reflux. To the hot solution was added 320 mL of acetic anhydride. The mixture was refluxed for 0.5 h and then poured onto ice. The solid was collected and washed twice with water and once with concentrated NH_4OH (this step converts any diacetate to monoacetate). The solid was then air-dried, dissolved in 1400 mL of boiling chloroform, treated with $MgSO_4$ and charcoal, and filtered while hot. The filtrate was boiled, and 500 mL of hexanes was added. The mixture was cooled in an ice bath. Solid was collected giving 145.9 g (65%) of the **11** as an orange solid: mp 167−168 °C; (ES+) *m*/*z* 222.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 2.28 (s, 3H), 3.96 (s, 3H), 7.22 (bs, 1H), 7.81 (s, 1H), 8.20 (s, 1H); Anal. ($C_{10}H_{12}N_2O_4$) C, H, N.

5-Ethoxy-2-methyl-4-nitroacetanilide (12). A mixture of 186 g (830 mmol) of **11** and 105.5 g (2.49 mol) of LiCl in 1115 mL of DMF was mechanically stirred at reflux for 12 h without cooling the condenser. The dark orange solution was cooled to room temperature and then left to stand overnight. To the stirring solution was added 114.65 g (830 mmol) of powdered K_2CO_3 and 265.4 mL (3.32 mol) of ethyl iodide. The mixture was slowly heated with stirring. At about 70 °C a rapid gas evolution ensues (probably ethyl chloride). After most gas had evolved, heating was continued at reflux temperature. The mixture was refluxed for 5 h and then poured onto ice water. The solid was collected, washed several times with water, and air-dried. The solid was dissolved in 2 L of boiling chloroform, treated with $MgSO_4$, and filtered while hot. The filtrate was boiled and diluted with 1.5 L of hexanes. After cooling, 105 g of **12** as a yellow solid (53%) was collected: 192−193 °C; (ES+) *m*/*z* 237.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H, J = 6.94 Hz), 2.15 (s, 3H), 2.22 (s, 3H), 4.19 (q, 2H, J = 6.95 Hz), 7.77 (s, 1H), 7.80 (s, 1H), 9.43 (s, 1H); Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

2-Acetylamino-4-ethoxy-5-nitrobenzoic Acid (13). A solution of 217.3 g of potassium permanganate and 75.2 g of magnesium sulfate in 5 L of water was heated to 80 °C, and 119 g (0.5 mol) of **12** was added in one portion. Heating at reflux was continued and after about 45 min, an additional 37.6 g of magnesium sulfate and then 108.7 g of potassium permanganate were added. After about 45 min of reflux, the reaction was filtered while hot. The manganese dioxide cake was reserved. Acidification of the filtrate with concentrated HCl gave product. The reserved manganese dioxide was boiled with 2 L of water and filtered. Acidification of the filtrate gave additional product. The combined product was collected by filtration and dried to give 68.19 g (50.8%) of **13**: mp 245−247 °C; (ES+) *m*/*z* 266.9 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, 3H, J = 8.51 Hz, 3H), 2.15 (s, 3H), 3.37 (bs, 1H), 4.13 (m, 2H), 8.50 (m, 2H), 11.53 (m, 1H); Anal. ($C_{11}H_{12}N_2O_6$) C, H, N.

3-Ethoxy-4-nitroaniline (14). To 600 mL of H_2O was slowly added 400 mL of H_2SO_4. To the hot mixture was added 118.5 g (44 mol) of **13**. The mixture was heated to 110−112 °C with stirring. Initially there was a vigorous gas evolution. After 1 h, the mixture was poured onto ice and was made basic with NH_4OH (CAUTION: exothermic!). The mixture cooled to room temperature, the solid was collected by filtration, washed several times with 500 mL portions of water, and then dried in a vacuum. The solid was extracted several times with warm EtOAc. The extracts were filtered and solvent was evaporated giving 57.8 g (71%) of **14**: mp = 111−114 °C; (ES+) *m*/*z* 183.17 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) 1.36 (t, 3H, J = 6.93 Hz), 4.06 (q, 2H, J = 6.94 Hz), 6.18 (dd, 1H, J = 2.19, J = 9.06 Hz), 6.25 (d, 1H, J = 2.16 Hz), 6.55 (bs, 1H), 7.80 (d, 1H, J = 9.09H); Anal. ($C_8H_{10}N_2O_3$) C, H, N.

7-Ethoxy-4-hydroxy-6-nitroquinoline-3-carbonitrile (9c). A mixture of 58.9 g (0.324 mol) of **14** and 77.2 g (0.456 mol) of ethyl (ethoxymethylene)cyanoacetate in 210 mL of toluene was refluxed for about 16 h. The reaction was cooled in an ice bath, and the product was filtered. This was washed with three portions of ether and then dried to give 94.3 g (95.8%) of the yellow intermediate **8f** as a mixture of cis/trans isomers which could be recrystallized, in 80% yield, from 2-methoxyethanol. A portion of this material (37.5 g, 0.123 mol) was added as a solid to 2.5 L of refluxing (256 °C) Dowtherm under N_2 in a 5 L three-necked flask equipped with a mechanical stirrer and a thermometer under nitrogen. The reaction mixture was stirred vigorously at this temperature for 1.25 h and then cooled to room temperature. The thick reaction mixture was diluted with 2 L of ether, filtered, and washed with ether to yield 24.2 g (76% from **8f**) of **9c** as an off-white solid. mp 244−247 °C; MS (ES+) *m*/*z* 259.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 8.76 (s, 1H), 8.52 (s, 1H), 7.25 (s, 1H), 4.29 (q, 2H, J = 6.9 Hz), 1.41 (t, 3H, J = 6.9 Hz); Anal. ($C_{12}H_9N_3O_4$) H, N; C: calcd, 55.60; found, 54.9.

4-Hydroxy-6-nitroquinoline-3-carbonitrile (9a). This compound was prepared from 10.0 g (38.3 mmol) of **7a** and ethyl (ethoxymethylene)cyanoacetate using the method described above for **9c** (80% yield): MS (ES+) *m*/*z* 216.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 13.3 (bs, 1H), 8.90 (s, 1H), 8.77 (d, 1H, J = 2.4 Hz), 8.51 (dd, 1H, J = 2.4, J = 9.0 Hz), 7.75 (d, 1H, J = 9.0 Hz); Anal. ($C_{10}H_5N_3O_3$) C, H, N.

7-Ethoxy-4-hydroxyquinoline-3-carbonitrile (9b). This compound was prepared from 10.0 g of **7b** and ethyl (ethoxymethylene)cyanoacetate using the method described above for **9c** giving a 63% yield of product after washing with hot EtOH: mp > 250 °C; MS (ES+) *m*/*z* 214.7 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.01 (d, 1H, J = 9.0 Hz), 7.02 (d, 1H, J = 9.0 Hz), 6.97 (d, 1H, J = 2.4 Hz), 4.15 (q, 2H, J = 6.9 Hz), 1.38 (t, 3H, J = 6.9 Hz); Anal. ($C_{12}H_{10}N_2O_2$) C, H, N.

Preparation of 9c by Nitration of 9b. A suspension of 5.0 g (23 mmol) of **9b** in 75 mL of TFA anhydride was stirred at room temperature as 6.7 g (70 mmol) of NH_4NO_2 was added portionwise over 6 h. Excess TFA anhydride was evaporated,

and the residue was stirred with 300 mL of H_2O. The solid was collected, dried, and washed with warm EtOH and dried giving 3.7 g (62%) of **9c**.

7-Methoxy-4-hydroxy-6-nitroquinoline-3-carbonitrile (9d). This compound was prepared from **7c** and ethyl (ethoxymethylene)cyanoacetate using the method described above for **9c**: MS (ES+) *m/z* 246 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 12.9 (bs, 1H), 8.80 (bs, 1H), 8.53 (s, 1H), 7.26 (s, 1H), 4.05 (s, 3H); Anal. ($C_{11}H_7N_3O_4$) C, H, N.

4-Hydroxy-8-methoxy-6-nitroquinoline-3-carbonitrile (9e). This compound was prepared from 12.6 g of **7d** and ethyl (ethoxymethylene)cyanoacetate using the method described above for **9c** giving a 65% yield of product as a brown solid after washing with hexanes: mp > 300 °C; MS (ES+) *m/z* 245.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 12.9 (bs, 1H), 8.65 (s, 1H), 8.44 (d, 1H, $J = 2.4$ Hz), 8.04 (d, 1H, $J = 2.4$ Hz), 4.14 (s, 3H); Anal. ($C_{11}H_7N_3O_4$) H, N; C: calcd. 53.88; found 55.19.

4-Hydroxy-7-trifluoromethoxyquinoline-3-carbonitrile (9f). This compound was prepared from 11.6 g of **7e** and ethyl (ethoxymethylene)cyanoacetate using the method described above for **9c** giving 7.9 g (48%) of product as a light brown solid after washing with hot EtOH: mp = 284−286 °C; MS (EI) *m/z* 254.03 $(M)^{+1}$

4-Hydroxy-6-nitro-7-trifluoromethoxyquinoline-3-carbonitrile (9g). Nitration of 5 g (19.7 mmol) of **9f** was accomplished using a method similar to that used in the preparation of **9c** giving 1.8 g (30%) of **9g** as a yellow solid: MS (EI) *m/z* 299.01 $(M)^{+1}$.

4-Chloro-7-ethoxy-6-nitroquinoline-3-carbonitrile (15b). In a 1 L round-bottomed flask, 20 g, (77 mmol) of **9b** was refluxed with 120 mL of $POCl_3$ under N_2 for 2.5 h. TLC (EtOAc: hexane 1:1) showed no starting material remaining. The excess $POCl_3$ was removed by rotary evaporation. The flask containing the solid residue was cooled in an ice bath, and 600 mL of CH_2Cl_2 was added to dissolve the residue. The resulting cold solution was added into a vigorously stirred solution of 250 mL ice-cold saturated K_2CO_3 and stirred for 30 min. The organic layer was separated, washed, dried ($MgSO_4$), and evaporated to give 18.6 g (87%) of compound **15b** as an off-white solid: mp 200−202 °C; MS (ES+) *m/z* 277.7 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.28 (s, 1H), 8.85 (s, 1H), 7.91 (s, 1H), 4.46 (q, 2H, $J = 6.9$ Hz), 1.41 (t, 3H, $J = 6.9$ Hz); Anal. ($C_{12}H_8ClN_3O_3$) C, H, N.

4-Chloro-6-nitroquinoline-3-carbonitrile (15a). This was prepared from 31.5 g of **9a** in 98% yield as described above: MS (CI) *m/z* 234.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H), 8.77 (d, 1H, $J = 2.7$ Hz), 8.52 (dd, 1H, $J = 2.7$, $J = 9.0$ Hz), 7.91 (d, 1H, $J = 9.0$ Hz).

4-Chloro-7-methoxy-6-nitroquinoline-3-carbonitrile (15c). This was prepared as described above from 2.1 g of **9c** in 15% yield after recrystallization from EtOAc: Anal. ($C_{11}H_6ClN_3O_3$) H, N; C: calcd 50.11, found 50.70.

4-Chloro-8-methoxy-6-nitroquinoline-3-carbonitrile (15d). This was prepared from 4.0 g of **9d** in 48% yield as described above. Product was recrystallized from EtOAc giving a tan solid: mp 230−234 °C; MS (ES+) *m/z* 263.7 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.35 (s, 1H), 8.06 (d, 1H, $J = 2.3$ Hz), 7.91 (d, 1H, $J = 2.3$ Hz), 4.16 (s, 3H).

4-Chloro-6-nitro-7-trifluoromethoxyquinoline-3-carbonitrile (15e). This was prepared from 1.5 g of **9e** in 78% yield as described above. The compound was obtained as a light brown solid.

4-(3-Chloro-4-fluorophenylamino)-7-ethoxy-6-nitroquinoline-3-carbonitrile (16e). A solution of 26.8 g (96.5 mmol) of **15b** and 14.05 g (96.5 mmol) of 3-chloro-4-fluoroaniline in 900 mL of 2-propanol was refluxed under N_2 for 3.5 h. TLC (EtOAc:hexane (1:1) showed no starting material remaining. After standing at room-temperature overnight, the solid was collected by filtration and washed with 2-propanol and ether giving **16e** 38.6 g (95%) as a yellow HCl salt.

4-(3-Bromophenylamino)-7-methoxy-6-nitroquinoline-3-carbonitrile (16c). This was prepared from 5.2 g of **15c** in 41% yield using the same method described above for **16e** except that the product was isolated as the free base by pouring the reaction mixture into saturated $NaHCO_3$, collecting and drying the solid, and purifying by chromatography: MS (ES+) *m/z* 399.0, 402.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.18 (bs, 1H), 9,12 (s, 1H), 8.73 (s, 1H), 7.65 (s, 1H), 7.38 (m, 4H), 4.08 (s, 3H); Anal. ($C_{17}H_{11}N_4BrO_3$) C, H, N.

4-(3-Bromophenylamino)-6-nitroquinoline-3-carbonitrile (16a). This was prepared from 4.2 g of **15a** in 44% yield using the same method described above for **16e.** The product was recrystallized from EtOAc: mp = 258−259 °C; MS (ES+) *m/z* 369.1, 371.0 $(M + H)^{+1}$; Anal. ($C_{16}H_9BrN_4O_2$) C, H, N.

4-(3-Bromophenylamino)-7-ethoxy-6-nitroquinoline-3-carbonitrile (16b). This was prepared from 2.1 g of **15b** in 83% yield using the same method described above for **16e**. Product was recrystallized from EtOAc: Anal. ($C_{18}H_{13}BrN_4O_3$) C, H, N.

4-(3-Chloro-4-fluorophenylamino)-6-nitroquinoline-3-carbonitrile (16d). This was prepared from 5.0 g of **15a** in 82% yield using the same method described above for **16e** to yield a light yellow solid: MS (ES+) *m/z* 343.1 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.57 (bs, 1H), 9.52 (s, 1H), 8.74 (s, 1H), 8.54 (dd, 1H, $J = 2.1$, $J = 9.0$ Hz), 8.07 (m, 1H), 7.67 (m, 1H), 7.54−7.41 (m, 2H); Anal. ($C_{16}H_8N_4ClFN_4O_2$) C, H, N.

4-(3-Chloro-4-fluorophenylamino)-7-methoxy-6-nitroquinoline-3-carbonitrile (16f). This was prepared from 4.4 g of **15c** in 48% yield using the same method described above for **16e**. The compound was isolated as the hydrochloride: Anal. ($C_{17}H_{10}Cl\ FN_4O_3$·HCl) C, H, N.

4-(3-Bromophenylamino)-8-methoxy-6-nitroquinoline-3-carbonitrile (16g). This was prepared from 1.9 g of **15d** in 80% yield using the same method described above for **16e**. Product was recrystallized from EtOAc giving a yellow solid: mp 163−165 °C; MS (ES+) *m/z* 398.8, 400.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.3 (bs, 1H), 9.07 (s, 1H), 8.73 (s, 1H), 7.94 (s, 1H), 7.36−7.44 (m, 4H), 4.07 (s, 3H); Anal. ($C_{11}H_7N_3O_4$) C, H, N.

4-(3-Chloro-4-fluorophenylamino)-6-nitro-7-trifluoromethoxyquinoline-3-carbonitrile (16h). This was prepared from **15e** in 100% yield using the same method described above for **16e.** The compound was obtained as a yellow solid: mp = 195−197 °C; MS (EI) *m/z* 426.01 $(M)^{+1}$.

6-Amino-4-(3-Chloro-4-fluoro-phenylamino)-7-ethoxyquinoline-3- carbonitrile (17e). The hydrochloride **16e** (38.6 g, 91.2 mmol) was mixed with 35.7 g (638 mmol) of iron powder. A solution of 43.9 g (820 mmol) of NH_4Cl in 280 mL of water was added followed by 985 mL of CH_3OH. The mixture was refluxed with mechanical stirring under N_2 for 4 h at which time TLC indicated complete reduction. The reaction mixture was filtered hot, and solids were washed with 500 mL of boiling CH_3OH. After the combined filtrate was evaporated, the residue was partitioned between 1.5 L of warm ethyl acetate and 700 mL of saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate, treated with activated charcoal, filtered, and evaporated to give a residue which was recrystallized from $CHCl_3$−hexanes giving 29.0 g (89%) of **17e** as a light greenish solid.

6-Amino-4-(3-bromophenylamino)quinoline-3-carbonitrile (17a). This was prepared from 4.0 g of **16a** in 100% yield as described for **17e**: MS (CI) *m/z* 339.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.34 (s, 1H), 8.40 (s, 1H), 7.73 (d, 1H, $J =$ 9.0 Hz), 7.45−6.97 (m, 5H), 5.80 (bs, 2H).

6-Amino-4-(3-bromophenylamino)-7-ethoxyquinoline-3-carbonitrile (17b). This was prepared from 2.5 g of **16a** in 59% yield as described for **17e**. Product was recrystallized from EtOAc giving a beige solid: mp 185−186 °C; MS (ES+) *m/z* 382.8, 384.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.24 (s, 1H), 8.48 (s, 1H), 7.14−7.25 (m, 5H), 6.96 (d, 1H, $J = 7.5$ Hz), 5.58 (d, 2H, $J = 4.8$ Hz), 4.26 (t, 2H, $J = 6.9$ Hz), 1.45 (t, 3H, $J =$ 6.9 Hz); Anal. ($C_{18}H_{15}BrN_4O$) C, H, N.

6-Amino-4-(3-bromophenylamino)-7-methoxyquinoline-3-carbonitrile (17c). This was prepared from 2.1 g of **16c** in 68% yield as described for **17e**. ^{1}H NMR (DMSO-d_6) δ 9.28 (s, 1H), 8.49 (s, 1H), 7.30 (s, 1H), 7.20 (m, 5H), 5.67 (bs, 2H), 4.00 (s, 3H); MS (ES+) *m/z* 369.1, 371.1 $(M + H)^{+1}$; Anal. ($C_{17}H_{13}N_4BrO$) C, H, N.

6-Amino-4-(3-Chloro-4-fluorophenylamino)quinoline-3-carbonitrile (17d). This was prepared from 5.4 g of **16d** in 91% yield as described for **17e**. Obtained as a yellow solid: MS (ES+) *m/z* 312.9 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.35 (bs, 1H), 8.34 (s, 1H), 7.70 (d, 1H, $J = 6.0$ Hz), 7.38 (m, 2H), 7.25 (d, 1H, $J = 6.0$ Hz), 7.15 (m, 2H), 5.76 (bs, 2H).

6-Amino-4-(3-Chloro-4-fluorophenylamino)-7-methoxyquinoline-3-carbonitrile (17f). This was prepared from 4.9 g of **16f** in 76% yield as described for **17e**. Product was recrystallized from EtOAc.

6-Amino-4-(3-bromophenylamino)-8-methoxyquinoline-3-carbonitrile (17g). This was prepared from 2.2 g of **16g** in 23% yield as described for **17e**. Product was recrystallized from EtOAc giving a yellow solid: mp 240−243 °C; MS (ES+) *m/z* 382.8, 384.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.21 (s, 1H), 8.34 (s, 1H), 7.21 (m, 3H), 6.99 (d, 1H, $J = 7.8$ Hz), 6.76 (d, 1H, $J = 2.1$ Hz), 6.65 (d, 2H, $J = 1.8$ Hz), 5.79 (d, 1H, $J = 4.5$ Hz), 3.91 (s, 3H); Anal. ($C_{17}H_{13}BrN_4O$) C, H; N: calcd, 15.17; found, 14.64.

6-Amino-4-(3-chloro-4-fluorophenylamino)-7-trifluoromethoxyquinoline-3-carbonitrile (17h). This was prepared from 1.0 g of **16h** in 96% yield as described for **17e**. The compound was obtained as a light brown solid: MS (EI) *m/z* 396.04, 384.8 $(M)^{+1}$.

4-(2-Methoxyethoxy)-but-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (21). A solution of 0.56 g (3.5 mmol) of 4-(2-methoxyethoxy)but-2-ynoic acid[23] and 0.46 g (3.4 mmol) of isobutyl chloroformate in 12 mL of THF was stirred at 0 °C under N_2 as 0.36 g (390 μL, 3.5 mmol) of *N*-methylmorpholine was added. After 15 min, 1.0 g (2.95 mmol) of solid **17a** was added. The mixture was stirred 3 h at 0° C and at room-temperature overnight. The mixture was poured into saturated $NaHCO_3$ and extracted several times with EtOAc. The organic extract was dried ($MgSO_4$). The solvent was evaporated, and the residue was chromatographed on silica gel. The column was initially eluted with $CHCl_3$−EtOAc 1:2 followed by EtOAc containing 0.3% CH_3OH to elute product. The solvent was removed from product fractions giving 0.53 g (39%) of **21** as a light yellow powder: MS (ES+) *m/z* 480.9 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 11.16 (s, 1H), 9.84 (s, 1H), 8.68 (m, 2H), 7.92 (m, 2H), 7.27 (m, 4H), 4.34 (s, 2H), 3.63 (m, 2H), 3.49 (m, 2H), 3.40 (m, 3H); Anal. ($C_{23}H_{19}N_4O_3$-Br·0.5H_2O) C, H, N, Br.

But-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (18). This compound was prepared from 2-butynoic acid and 1.5 g of **17a** in 36% yield using the method described above for **21**. The compound was purified by boiling in EtOH giving a light yellow solid: mp 283−285 °C; HRMS (CI) *m/z* 404.0274 $(M + H)^{+1}$, $\Delta = -0.1$ mmu; ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H), 9.84 (bs, 1H), 8.73 (s, 1H), 8.64 (bs, 1H), 7.31 (m, 1H), 7.80−7.90 (m, 2H), 7.29 (m, 2H), 7.18 (m, 1H), 2.08 (s, 3H); Anal. ($C_{20}H_{13}BrN_4O$ −0.5 H_2O) C, H, N.

Methoxybut-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyano-quinolin-6-yl]amide (19). This compound was prepared from 4-methoxybut-2-ynoic acid and 50 mg of **17a** in 36% yield using the method described above for **21**: MS (ES+) *m/z* 435.1 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 11.3 (s,1H), 10.08 (s, 1H), 8.71 (s, 1H), 8.64 (bs, 1H), 7.89 (m. 2H), 7.31 (m, 4H), 4.37 (s, 2H), 3.35 (2, 3H); Anal. ($C_{21}H_{15}BrN_4O_2$·0.5H_2O) C, H, N.

4-Methoxymethoxybut-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (20). This compound was prepared from 4-methoxymethoxybut-2-ynoic acid[23] and 1.0 g of **17a** in 48% yield using the method described above for **21** as a light yellow powder: MS (ES+) *m/z* 465.1, 467.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 11.18 (s, 1H), 9.84 (s, 1H), 8.65 (m, 2H), 7.92 (m, 2H), 7.30 (m 4H), 4.70 (s, 2H), 4.60 (s, 2H), 3.32 (s, 3H); Anal. ($C_{22}H_{17}N_4O_3Br$·0.4H_2O) C, H, N.

4-Thiomorpholin-4-yl-but-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (22). This compound was prepared from 4-(4-thiomorpholinyl)-2-butynoic acid[23] and 1.0 g of **17a** in 32% yield using the method described above for **21.** Purification by chromatography using EtOAc−CH_3OH (98:2) gave a light brown solid: HRMS (EI) *m/z* 505.0560 (M^{+1}), $\Delta = 1.2$ mmu; ^{1}H NMR (DMSO-d_6) δ 11.08 (bs, 1H), 9.83 (bs, 1H), 8.69 (bs, 1H), 8.66 (s, 1H), 7.96 (m, 2H), 7.41 (bs, 1H), 7.30 (m, 2H), 7.12 (m, 1H), 3.57 (s, 2H), 2.76 (m, 4H), 2.64 (m, 4H); Anal. ($C_{24}H_{20}N_5OSBr$·0.6H_2O) C, H; N: calcd, 13.54; found, 13.02.

4-((2S)-2-Methoxymethyl-pyrrolidin-1-yl)but-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]-amide (23). This compound was prepared from 4-((2*S*)-2-methoxymethylpyrrolidin-1-yl)but-2-ynoic acid[23] and 1.0 g of **17a** in 37% yield using the method described above for **21**. Purification was by chromatography using EtOAc−CH_3OH (9:1): HRMS (CI) *m/z* 518.1178 $(M + H)^{+1}$, $\Delta = 1.4$ mmu: ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 9.83 (s, 1H), 8.69 (bs, 1H), 8.66 (s, 1H), 7.96 (m, 2H), 7.41−7.19 (m, 4H), 3.71 (m, 2H), 3.34 (s, 3H), 3.26 (m, 4H), 1.89 (m, 2H), 1.70 (m, 2H), 1.49 (m, 1H); Anal. ($C_{26}H_{24}BrN_5O_2$-2.75 H_2O) C, H, N; N: calcd, 10.13; found, 11.72.

4-Dipropylaminobut-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (24). This compound was prepared from 4-dipropylaminobut-2-ynoic acid[23] and 1.0 g of **17a** in 51% yield using the method described above for **21**. This was purified by chromatography using EtOAc to give a light yellow solid: ^{1}H NMR (DMSO-d_6) δ 10.99 (s, 1H), 9.83 (bs, 1H), 8.68 (s, 1H), 8.65 (bs, 1H), 7.92 (m, 2H), 7.39−7.19 (m, 4H), 3.61 (s, 2H), 2.46 (m, 4H), 1.45 (m, 4h), 0.87 (t, 6H, $J = 7.3$ Hz); Anal. ($C_{26}H_{26}BrN_5O$·0.33H_2O) C, H, N.

4-Diisopropylaminobut-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (25). This compound was prepared from 4-diisopropylaminobut-2-ynoic acid [23] and 1.5 g of **17a** in 48% yield using the method described above for **21.** The compound was purified by silica gel chromatography using EtOAc: MS (ES+) *m/z* 504.1 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H), 9.82 (s, 1H), 8.69 (s, 1H), 8.65 (s, 1H), 7.94 (m, 2H), 7.40 (bs, 1H), 7.31 (m, 2H), 7.19 (bs, 1H), 3.59 (s, 2H), 3.34 (m, 12H), 3.16 (m, 2H); Anal. ($C_{18}H_{15}$-BrN_4O·0.5H_2O) C, H, N.

But-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide (26). This compound was prepared from 2-butynoic acid[23] and **17b** using the method described above for **21**: MS (ES+) *m/z* 437.1, 438.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.07 (s, 1H), 9.76 (s, 1H), 8.68 (bs, 1H), 8.62 (s, 1H), 7.45 (m 5H), 4.01 (s, 3H), 2.05 (s, 3H); Anal. ($C_{21}H_{15}BrN_4O_2$) C, H, N.

4-Methoxybut-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide (27). This compound was prepared from 4-methoxybut-2-ynoic acid[23] and 0.26 g of **17b** in 42% yield using the method described above for **21**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (97:3) giving a light yellow solid: HRMS (CI) *m/z* 465.0552 $(M + H)^{+1}$, $\Delta = 1.0$ mmu; ^{1}H NMR (DMSO-d_6) δ 10.39 (bs, 1H), 9.71 (s, 1H), 8.67 (bs, 1H), 8.63 (s, 1H), 7.47 (s, 1H), 7.40−7.21 (m, 4H), 4.35 (s, 2H), 4.02 (s, 3H); Anal. ($C_{22}H_{17}BrN_4O_3$-1.0 H_2O) C, H, N.

4-Dimethylaminobut-2-ynoic Acid [4-(3-Bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide (28). This compound was prepared from 4-(dimethylamino)but-2-ynoic acid[23] and 1.0 g of **17b** in 14% yield using the method described above for **21**. The compound was obtained as a beige solid: mp = 120 °C (dec); MS (ES+) *m/z* 480.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.24 (s, 1H), 9.74 (s, 1H), 8.66 (s, 1H), 8.63 (s, 1H), 7.46 (s, 1H), 7.39 (s, 1H), 7.31 (m, 2H), 7.20 (d, 1H, $J =$ 3.3 Hz), 4.02 (s, 3H), 3.46 (s, 2H), 2.25 (s, 6H); HRMS (ES+) *m/z* 477.0800, $\Delta = 0.9$ mmu.

4-Dimethylaminobut-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-ethoxyquinolin-6-yl]amide (5). To 15 g (63.2 mmol) of trimethylsilyl 4-bromo-2-butenoate[27] in 36 mL of CH_2Cl_2, was added 8.8 g (69.5 mmol) of oxalyl chloride, followed by 1 drop of DMF. The solution was stirred for 2 h during which time gas evolution ceased. The solvent was evaporated giving the carboxylic acid chloride **29a**. A solution of 19.6 g (54.9 mmol) of **17e** and 11.5 mL (65.9 mmol) of *N,N*-diisopropylethylamine in 366 mL of anhydrous THF was stirred under N_2 in an ice bath as a solution of the acid chloride prepared above in 183 mL of THF was added over 15

min. The reaction vessel was sealed and stored in the freezer overnight. The solution was evaporated, and the residue was partitioned between saturated $NaHCO_3$ and EtOAc. The organic layer was separated, washed, dried ($MgSO_4$), and passed through a thin layer of silica gel to give 32 g of the crude product as an orange solid. The solid was refluxed with 400 mL of CH_3OH for 0.5 h. After being cooled to room temperature, the solid was collected and washed with CH_3OH followed by hexane to give 21.3 g (77%) of 4-bromobut-2-enoic acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-ethoxyquinolin-6-yl]amide as a beige solid. Some additional product could be isolated from the mother liquor. This compound was contaminated with about 20−25% of the corresponding chloro derivative.

A 19.9 g (39.53 mmol) portion of the bromo/chloro mixture prepared above was dissolved in 800 mL of THF and cooled to 0 °C. A solution of 2 M $(CH_3)_2NH$ (39.5 mL, 79.07 mmol) in THF was added in one portion. The reaction solution was stirred at room-temperature overnight. Another 1 equiv (19.8 mL) of $(CH_3)_2NH$ solution was added. After being stirred overnight at room temperature, the reaction solution was evaporated, and the residue was partitioned between EtOAc and saturated K_2CO_3. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give 17 g of orange glass. The crude product was taken up in acetone and purified by column chromatography eluting with acetone. The product fractions were pooled and evaporated to give 9.8 g of a yellow glass. This was dissolved in 350 mL of hot EtOAc and then concentrated to a small volume. A few drops of methanol were added to assist crystallization. After standing at room-temperature overnight, the beige crystals were collected to give 7.09 g (39%) of **5.** Additional product could be isolated from the mother liquor: mp 196−198; MS (ES+) *m/z* 468; ^{1}H NMR (DMSO-d_6) δ 9.73 (bs, 1H), 9.61 (s, 1H), 9.00 (s, 1H), 8.54 (s, 1H), 7.41 (m, 3H), 7.24 (m, 1H), 6.83 (dt, 1H, J = 5.6, J = 15.4 Hz), 4.32 (q, 2H, J = 7.0 Hz), 3.07 (d, 2H, J = 5.1 Hz), 1.48 (t, 3H, J = 7.0 Hz); Anal. ($C_{24}H_{23}ClFN_5O_2$) C, H, N

4-Dimethylaminobut-2-enoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (30). This was prepared from 2.54 g of **17a** in 58% yield using the method described above for **5**. The product was purified by recrystallization from methyl Cellosolve: MS (ES+) *m/z* 449.9, 451.9; ^{1}H NMR (DMSO-d_6) δ 2.19 (s, 6H), 3.09 (d, J = 4.86, 2H), 6.34 (d, J = 15.5 Hz 1H), 6.80 (tt, J = 5.85, J = 15.3 Hz, 1H) 7.19 (s, 1H), 7.29 (m, 2H), 7.40 (s, 1H), 7.94 (m, 2H), 8.64 (s, 1H), 8.82 (s, 1H), 9.82 (s, 1H) 10.48 (s, 1H); Anal. ($C_{22}H_{20}BrN_5O\cdot 1.0H_2O$) C, H, N.

4-Dimethylaminobut-2-enoic Acid [4-(3-Bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide (31). This was prepared from 1.9 g of **17c** in 68% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH 3:2 as a beige solid: mp = 120−123 °C (dec); MS (ES+) *m/z* 480.0, 481.9 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.72 (s, 1H), 9.67 (s, 1H), 9.03 (s, 1H), 8.62 (s, 1H), 7.46 (s, 1H), 7.36 (s, 1H), 7.28 (m, 2H), 7.16 (m, 1H), 6.77 (m, 1H), 6.61 (d, 1H, J = 11.4 Hz), 4.05 (s, 3H), 3.06 (d, 2H, J = 4.3 Hz), 2.17 (s, 6H); Anal. ($C_{23}H_{22}BrN_5O_2\cdot 1.2H_2O$) C, H, N.

4-Morpholin-4-yl-but-2-enoic Acid [4-(3-Bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide (32). The acid chloride **29a** was prepared and reacted with **17c** as described above in the preparation of **5** from **17e**. To the solution of 0.95 g of crude 4-bromobut-2-enoic acid [4-(3-bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide in THF was added 30 equivalents of morpholine. After standing overnight, the reaction solution was evaporated and the residue was partitioned between EtOAc and saturated K_2CO_3. The organic layer was dried ($MgSO_4$), filtered, and evaporated. The product was purified by silica gel chromatography eluting with EtOAc−CH_3OH (85:15) giving 0.38 g (39%) of product as a light gray solid: MS (ES+) *m/z* 521.9, 523.8 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.72 (s, 1H), 9.70 (s, 1H), 8.79 (d, 1H, J = 3.2 Hz), 8.61 (s, 1H), 7.46 (s, 1H), 7.36 (s, 1H), 7.29 (m, 2H), 7.16 (m, 1H), 6.75 (m, 1H), 6.63 (d, 1H, J = 15 Hz), 4.05 (s, 3H), 3.60 (t, 4H, J = 4.5 Hz), 3.15 (d, 2H, J = 5.1 Hz), 2.38 (t, 4H, J = 4.5 Hz); Anal. ($C_{25}H_{24}BrN_5O_3\cdot 1.0H_2O$) C, H; N: calcd, 12.96; found 12.14.

4-Dimethylaminobut-2-enoic Acid [4-(3-Bromophenylamino)-3-cyano-7-ethoxyquinolin-6-yl]amide (33). This was prepared from 0.63 g of **17b** in 54% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (7:3) giving a tan solid: MS (ES+) *m/z* 494.0, 496.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.22 (s, 1H), 9.52 (s, 1H), 9.02 (d, 1H, J = 3.1 Hz), 8.61 (s, 1H), 7.44 (s, 1H), 7.36 (s, 1H), 7.28 (m, 2H), 7.18 (m, 1H), 6.77 (m, 1H), 6.62 (d, 2H, J = 14.7 Hz), 4.33 (q, 2H, J = 7.0 Hz), 3.07 (d, 2H, J = 5.0 Hz), 2.17 (s, 6H), 1.48 (t, 3H, J = 7.0 Hz); Anal. ($C_{24}H_{24}BrN_5O_2\cdot 0.7H_2O$) C, H, N.

4-Morpholin-4-yl-but-2-enoic Acid [4-(3-Bromophenylamino)-3-cyano-7-ethoxyquinolin-6-yl]amide (34). This was prepared from 0.63 g of **17b** in 57% yield using the method described above for **32**. Product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH 7:3 giving a white solid: mp = 199−203 °C; MS (ES+) *m/z* 535.9, 538.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.72 (s, 1H), 9.52 (s, 1H), 9.00 (d, 1H, J = 3.3 Hz), 8.59 (s, 1H), 7.44 (s, 1H), 7.35 (s, 1H), 7.28 (m, 2H), 7.16 (m, 1H), 6.76 (m, 1H), 6.62 (d, 1H, J = 16.5 Hz), 4.33 (q, 2H, J = 6.9 Hz), 3.60 (t, 4H, J = 4.5 Hz), 3.14 (d, 2H, J = 5.3 Hz), 2.40 (t, 4H, J = 4.5 Hz), 1.48 (t, 3H, J = 6.9 Hz); Anal. ($C_{26}H_{26}BrN_5O_3$) C, H, N.

4-Dimethylaminobut-2-enoic Acid [4-(3-Bromophenylamino)-3-cyano-8-methoxyquinolin-6-yl]amide (35). This was prepared from 0.49 g of **17b** in 45% yield using the method described above for **5**. Product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH 7:3: mp = 219 °C (dec); MS (ES+) *m/z* 480.0, 482.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.43 (s, 1H), 9.69 (s, 1H), 8.56 (s, 1H), 8.28 (s, 1H), 7.53 (s, 1H), 7.30 (m, 3H), 7.13 (m, 1H), 6.81 (m, 1H), 6.31 (d, 1H, J = 15.6 Hz), 3.95 (s, 3H), 3.10 (d, 2H, J = 4.2 Hz), 2.12 (s, 6H); Anal. ($C_{23}H_{22}BrN_5O_2\cdot 2.0H_2O$) C, H; N: calcd, 13.56; found, 13.05.

4-Morpholin-4-yl-but-2-enoic Acid [4-(3-Bromophenylamino)-3-cyano-8-methoxyquinolin-6-yl]amide (36). This was prepared from 0.49 g of **17g** in 45% yield using the method described above for **32**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (7:3): mp = 220 °C (dec); MS (ES+) *m/z* 524.0 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 9.67 (s, 1H), 8.60 (s, 1H), 8.29 (d, 1H, J = 1.5 Hz), 7.50 (d, 1H, J = 1.5 Hz), 7.25−7.36 (m, 3H), 7.14 (m, 1H), 6.81 (m, 1H), 6.33 (d, 1H, J = 15.3 Hz), 3.98 (s, 3H), 3.61 (m, 4H), 3.15 (d, 2H, J = 5.7 Hz), 2.40 (s, 6H); Anal. ($C_{25}H_{24}BrN_5O_2\cdot 1.0H_2O$) C, H, N.

4-Dimethylaminobut-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyanoquinolin-6-yl]amide (37). This was prepared from 1.5 g of **17d** in 21% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (3:7) giving a light yellow solid: HRMS (EI) *m/z* 423.1268 M^{+1}, Δ = −0.6 mmu; ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H), 9.94 (bs, 1H), 8.82 (s, 1H), 8.53 (s, 1H), 7.89 (s, 1H), 7.43 (m, 2H), 7.23 (m, 1H), 6.83 (dt, 1H, J = 5.7, J = 15.2 Hz), 6.34 (d, 1H, J = 15.3 Hz), 3.08 (d, 2H, J = 5.7 Hz), 2.18 (s, 6H); Anal. ($C_{22}H_{19}ClFN_5O\cdot 0.5\ H_2O$) C, H, N.

4-Morpholin-4-yl-but-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyanoquinolin-6-yl]amide (38). This was prepared from 1.5 g of **17d** in 36% yield using the method described above for **32**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (9:1) giving a light yellow solid:^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H), 9.84 (s, 1H), 8.82 (s, 1H), 8.58 (s, 1H), 7.92 (m, 2H), 7.46 (m, 2H), 7.28 (m, 1H), 6.80 (dt, 1H, J = 5.7, J = 15.6 Hz), 6.35 (d, 1H, J = 15.6 Hz), 3.61 (t, 4H, J = 4.6 Hz), 3.15 (d, 2H, J = 5.7 Hz), 2.46 (m, 4H); Anal. ($C_{24}H_{21}ClFN_5O_2\cdot 1.5H_2O$) C, H, N.

4-Dimethylaminobut-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-methoxyquinolin-6-yl]-amide (39). This was prepared from 1.5 g of **17f** in 61% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with EtOAc−

CH_3OH 7:3: mp = 206−208 °C; MS (ES+) *m/z* 454.0 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.74 (s, 1H), 9.67 (s, 1H), 9.00 (s, 1H), 8.56 (s, 1H), 7.43 (m, 3H), 7.25 (m, 1H), 6.77 (m, 1H), 6.60 (d, 1H, J = 15.4 Hz), 4.04 (s, 3H), 3.06 (d, 2H, J = 5.7 Hz), 2.17 (s, 6H); Anal. ($C_{23}H_{21}ClFN_5O_2 \cdot 0.2H_2O$) C, H, N.

4-Diethylaminobut-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-methoxyquinolin-6-yl]amide (40). This was prepared from 1.1 g of **17f** and diethylamine in 40% yield using the method described above for **5**. Product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (4:1): mp = 173−175 °C; MS (ES+) *m/z* 482.0 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.73 (s, 1H), 9.67 (s, 1H), 9.00 (s, 1H), 8.56 (s, 1H), 7.43 (m, 3H), 7.28 (m, 1H), 6.77−6.85 (m, 1H), 6.60 (d, 1H, J = 12 Hz), 4.02 (s, 3H), 3.30 (d, 2H, J = 7.0 Hz), 2.46 (q, 4H, J = 6.9 Hz), 0.98 (t, 6H, J = 6.9 Hz); Anal. ($C_{25}H_{25}ClFN_5O_2 \cdot 1.6H_2O$) C, H, N.

4-Morpholin-4-yl-but-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-methoxyquinolin-6-yl]-amide (41). This was prepared from 1.2 g of **17f** in 48% yield using the method described above for **32**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (7:3) giving a white solid: mp = 195−200 °C; MS (ES+) *m/z* 496.0 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.74 (s, 1H), 9.69 (s, 1H), 9.00 (d, 1H, J = 3.6 Hz), 8.55 (s, 1H), 7.41 (m, 3H), 7.24 (m, 1H), 6.77 (m, 1H), 6.62 (d, 1H, J = 15.0 Hz), 4.04 (s, 3H), 3.60 (t, 4H, J = 4.4 Hz), 3.13 (d, 2H, J = 5.3 Hz), 2.36 (t, 4H, J = 4.4 Hz); Anal. ($C_{25}H_{23}ClFN_5O_3 \cdot 0.5H_2O$) C, H, N.

4-Dimethylaminobut-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-trifluoromethoxyquinolin-6-yl]amide (42). This was prepared from 0.77 g of **17h** in 42% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with eluting with EtOAc−MeOH−Et_3N (80:8:1): ^1H NMR (DMSO-d_6) δ 10.16 (s, 1H), 10.0 (bs, 1H), 9.00 (s, 1H), 8.61 (s, 1H), 7.99 (d, 1H, J = 1.5 Hz), 7.58 (dd, 1H, J = 6.6, J = 2.4 Hz), 7.50 (t, 1H, J = 9.0 Hz), 7.33 (m, 1H), 6.82 (td, 1H, J = 6.0, J = 15.3 Hz), 6.53 (d, 1H, J = 15.3 Hz), 3.10 (d, 1H, J = 6 Hz), 2.18 (s, 6H); HRMS (ES+) *m/z* 508.1162, Δ = 0.1 mmu: Anal. ($C_{23}H_{18}ClF4N_5O_2 \cdot 1.0H_2O$) C, H; N: calcd, 13.32; found, 12.79.

4-Dimethylamino-pent-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-methoxyquinolin-6-yl]-amide (43). This was prepared from 0.5 g of **17f** in 46% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (1:4) giving a light yellow solid: HRMS (EI) *m/z* 467.1487 M^{+1}, Δ = 3.7 mmu; ^1H NMR (DMSO-d_6) δ 9.67 (s, 1H), 9.00 (s, 1H), 8.52 (s, 1H), 7.40 (s, 3H), 7.22 (s, 1H), 6.74 (dd, 1H, J = 7.2, J = 18.3 Hz), 6.54 (d, 1H, J = 18.3 Hz), 4.03 (s, 3H), 3.15 (m, 1H), 2.17 (s, 6H), 1.18 (d, 3H, J = 6.6 Hz); Anal. ($C_{24}H_{23}ClFN_5O_2 \cdot 1.0H_2O$) C, H, N.

4-Dimethylamino-pent-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-methoxyquinolin-6-yl]-amide (44). This was prepared from 0.7 g of **17f** in 26% yield using the method described above for **5**. The product was purified by chromatography on silica gel eluting with EtOAc−CH_3OH (3:2) giving a light yellow solid: HRMS (EI) *m/z* 495.1843 M^{+1}, Δ = 0.6 mmu; ^1H NMR (DMSO-d_6) δ 9.77 (bs, 1H), 9.66 (s, 1H), 9.01 (s, 1H), 8.53 (s, 1H), 7.43 (m, 3H), 7.22 (m, 1H), 6.89 (dd, 1H, J = 6.3, J = 15.6 Hz), 6.53 (d, 1H, J = 15.6 Hz), 4.01 (s, 3H), 3.53 (m, 1H), 2.45 (m, 4H), 1.12 (d, 3H, J = 6.7 Hz), 0.98 (t, 6H, J = 7.1 Hz); Anal. ($C_{26}H_{27}ClFN_5O_2 \cdot 1.0H_2O$) C, H, N.

4-Morpholin-4-yl-pent-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-methoxyquinolin-6-yl]-amide (45). This was prepared from 1.02 g of **17f** in 35% yield using the method described above for **32**. The product was purified by prep TLC chromatography on silica gel eluting with EtOAc−CH_3OH (85:15) giving a light yellow solid: HRMS (EI)-*m/z* 509.1659 M^{+1}, Δ = −2.9 mmu; ^1H NMR (DMSO-d_6) δ 9.74 (s, 1H), 9.72 (s, 1H), 8.96 (s, 1H), 8.56 (s, 1H), 7.45 (m, 3H), 7.28 (m, 1H), 6.78 (dd, 1H, J = 6.9, J = 15.3 Hz), 6.55 (d, 1H, J = 15.3 Hz), 4.04 (s, 3H), 3.58 (m, 4H), 3.14 (m, 1H), 2.44 (m, 4H), 1.14 (d, 3H, J = 6.6 Hz); Anal. ($C_{26}H_{25}ClFN_5O_3 \cdot 3.0H_2O$) C, H, N.

***N*-[4-(3-Bromophenylamino)-3-cyano-7-methoxyquinolin-6-yl]acrylamide (48).** A solution of 1.5 g (4.1 mmol) of **17c** and 0.41 g (4.1 mmol) of *N*-methylmorpholine in 30 mL of THF was stirred in an ice bath under N_2 as 0.42 g (447 μL, 4.7 mmol) of acryloyl chloride was added over 5 min. After 1 h, the mixture was poured into saturated $NaHCO_3$ and extracted with EtOAc. The extract was dried ($MgSO_4$), and the solvent was removed. The residue was chromatographed on silica gel eluting with $CHCl_3$−EtOAc mixtures giving 0.5 g (29%) of **48** as a light yellow solid: MS (ES+) *m/z* 423.1, 425.1 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.80 (bs, 1H), 9.74 (bs, 1H), 9.03 (s, 1H), 8.63 (s, 1H), 7.48 (s, 1H), 7.27 (m, 4H), 6.81 (dd, 1H, J = 10.2 Hz, J = 16.9 Hz), 6.29 (d, 1H, J = 16.9 Hz), 5.80 (d, 1H, J = 10.2 Hz), 4.06 (s, 3H); Anal. ($C_{20}H_{15}N_4O_2Br \cdot 0.4H_2O$) C, H, N.

***N*-[4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]acrylamide (47).** This was prepared from acryloyl chloride and 1.0 g of **17a** as described above for **48**. After recrystallization from EtOH, a 50% yield of product was obtained: HRMS (EI) *m/z* 392.0268 $(M)^{+1}$, Δ = 0.5 mmu; ^1H NMR (DMSO-d_6) δ 10.6 (s, 1H), 9.83 (bs, 1H), 8.82 (s, 1H), 8.64 (s, 1H), 7.96 (s, 2H), 7.40 (m, 1H), 7.31 (m, 2H), 7.15 (m, 1H), 6.50 (m, 1H), 6.30 (m, 1H), 5.83 (m, 1H); Anal. ($C_{19}H_{13}BrN_4O \cdot 0.5H_2O$) C, H, N.

(*E*)-But-2-enoic Acid [4-(3-Bromophenylamino)-3-cyanoquinolin-6-yl]amide (49). This was prepared from 2-butenoyl chloride and 1.0 g of **17a** as described above for **48.** After boiling in EtOH, an 80% yield was obtained as a solid: HRMS (CI) *m/z* 407.0493 $(M + H)^{+1}$, Δ = 1.5 mmu; ^1H NMR (DMSO-d_6) δ 10.38 (s, 1H), 9.84 (bs, 1H), 8.79 (s, 1H), 8.63 (s, 1H), 7.94 (m, 2H), 7.39 (m, 1H), 7.29 (m, 2H), 7.18 (m, 1H), 6.83 (m, 1H), 6.20 (d, 1H, J = 15.1 Hz), 0.94 (d, 3H, J = 6.9 Hz); Anal. ($C_{20}H_{15}BrN_4O$-0.5 H_2O) C, H, N.

(*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)-3-cyano-7-ethoxy-6-quinolinyl]-2-butenamide (50). This was prepared from 2-butenoyl chloride and 0.71 g of **17e** as described above for **48.** After chromatography on silica gel eluting with CH_2Cl_2−EtOAc (3:1), a 48% yield of **50** was obtained as a white solid: MS (ES+) *m/z* 424.9, 426.9 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.72 (s, 1H), 9.34 (s, 1H), 8.98 (s, 1H), 8.54 (s, 1H), 7.41 (s, 1H), 7.24 (m, 1H), 7.43 (m, 2H), 6.84 (dt, 1H), 6.47 (dd, 1H), 4.32 (q, 2H), 1.89 (dd, 3H), 1.48 (t, 3H); Anal. ($C_{22}H_{18}ClFN_4O_2$) C, H, N.

***N*-[4-(3-Chloro-4-fluoroanilino)-3-cyano-7-ethoxy-6-quinolinyl]-2-[(dimethylamino)methyl]acrylamide (52).** To a suspension of 1.65 g (10 mmol) 2-dimethylaminomethylacrylic acid hydrochloride[28] in 25 mL of CH_3CN was added 0.87 mL (1.27 g, 10 mmol) of oxalyl chloride followed by about 1 μL of DMF under a N_2 atmosphere. The mixture was stirred at 65 °C for 1 h. The solution was concentrated at reduced pressure to about 12 mL and cooled in an ice bath under N_2. To this solution was added dropwise a solution of 1.78 g (5 mmol) **17e** in 10 mL of *N*-methylpyrrolidinone. After stirring 1 h, the mixture was poured into a solution of $NaHCO_3$. The resulting solid was collected by filtration and dried in a vacuum. The residue was chromatographed on silica gel eluting with CH_2Cl_2−CH_3OH (97:3). Product fractions were combined giving 0.66 g (28%) of **52** as a light yellow solid: mp 132−135 °C; MS (ES+) *m/z* 449.9, 451.9; ^1H NMR (DMSO-d_6) δ 2.19 (s, 6H), 3.09 (d, J = 4.86, 2H), 6.34 (d, 1H, J = 15.5 Hz), 6.80 (tt, 1H J = 5.85, J = 15.3, Hz) 7.19 (s, 1H), 7.29 (m, 2H), 7.40 (s, 1H), 7.94 (m, 2H), 8.64 (s, 1H), 8.82 (s, 1H), 9.82 (s, 1H) 10.48 (s, 1H); Anal. ($C_{24}H_{23}ClFN_5O_2 \cdot 0.75H_2O$) C, H, N.

***N*-[4-(3-Chloro-4-fluorophenylamino)-3-cyanoquinolin-6-yl]-2-morpholin-4-ylmethylacrylamide (51).** This was prepared from 2-(morpholin-4-yl)methylacrylic acid hydrochloride[28] and 1.0 g of **17e** as described above for **52**. It was purified by chromatography eluting with EtOAc−CH_3OH 9:1 giving a light yellow solid: HRMS (EI) *m/z* 465.1361 M^{+1}, Δ = 0.1 mmu; ^1H NMR (DMSO-d_6) δ 11.20 (s, 1H), 9.85 (s, 1H), 8.98 (s, 1H), 8.59 (s, 1H), 7.94 (m, 2H), 7.51 (m, 3H), 7.29 (m, 1H), 6.12 (s,

1H), 5.69 (s, 1H), 3.64 (m, 2H), 2.50 (m, 4H); Anal. ($C_{24}H_{21}ClFN_5O_2 \cdot 0.5H_2O$) C, H, N.

4-Trimethylammonium-but-2-enoic Acid [4-(3-Chloro-4-fluorophenylamino)-3-cyano-7-ethoxyquinolin-6-yl]-amide Iodide (53). To a warm solution of 0.52 g (1.1 mmol) of **5** in 10 mL of THF was added 0.17 g (1.2 mmol) of CH_3I. After standing overnight in the refrigerator, the solution was diluted with EtOAc. Solid was collected and washed with EtOAc and ether giving 0.63 g (93%) of **53** as an off-white powder: MS (ES+) *m/z* 482.2 (M^{+1}).

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