Optimization of 6,7-Disubstituted-4-(arylamino)quinoline-3-carbonitriles as Orally Active, Irreversible Inhibitors of Human Epidermal Growth Factor Receptor-2 Kinase Activity

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A series of new 6,7-disubstituted-4-(arylamino)quinoline-3-carbonitrile derivatives that function as irreversible inhibitors of human epidermal growth factor receptor-2 (HER-2) and epidermal growth factor receptor (EGFR) kinases have been prepared. These compounds demonstrated enhanced activities for inhibiting HER-2 kinase and the growth of HER-2 positive cells compared to our EGFR kinase inhibitor **86** (EKB-569). Three synthetic routes were used to prepare these compounds. They were prepared mostly by acylation of 6-amino-4-(arylamino)quinoline-3-carbonitriles with unsaturated acid chlorides or by amination of 4-chloro-6-(crotonamido)-quinoline-3-carbonitriles with monocyclic or bicyclic anilines. The third route was developed to prepare a key intermediate, 6-acetamido-4-chloroquinoline-3-carbonitrile, that involved a safer cyclization step. We show that attaching a large lipophilic group at the para position of the 4-(arylamino) ring results in improved potency for inhibiting HER-2 kinase. We also show the importance of a basic dialkylamino group at the end of the Michael acceptor for activity, due to intramolecular catalysis of the Michael addition. This, along with improved water solubility, resulted in compounds with enhanced biological properties. We present molecular modeling results consistent with the proposed mechanism of inhibition. Binding studies of one compound, **25o** (C-14 radiolabeled), showed that it binds irreversibly to HER-2 protein in BT474 cells. Furthermore, it demonstrated excellent oral activity, especially in HER-2 overexpressing xenografts. Compound **25o** (HKI-272) was selected for further studies and is currently in phase I clinical trials for the treatment of cancer.

Introduction

Protein tyrosine kinases play a key role in signal transduction pathways that regulate numerous cellular functions including proliferation, differentiation, migration, and angiogenesis. A large number of receptor and nonreceptor tyrosine kinases have been identified. Among them, the epidermal growth factor receptor (EGFR) tyrosine kinase (known as EGFR, HER-1, or ErbB-1) and human epidermal growth factor receptor-2 (known as HER-2, ErbB-2 or neu) have been widely studied as drug discovery targets. They are two of the four members of the EGF receptor family that also includes HER-3 (ErbB-3) and HER-4 (ErbB-4). These four closely related proteins share common structural elements such as an extracellular ligand binding domain and an intracellular tyrosine kinase domain. At least 12 different ligands that bind to the EGFR, HER-3, and HER-4 receptors have been identified. However, there is no known ligand for HER-2. Upon ligand binding, these receptors dimerize with themselves or with other family members and undergo autophosphorylation of tyrosine residues within the C-terminal cytoplasmic domain. These phosphorylated residues serve as docking sites for recruitment of downstream signaling proteins, leading to subsequent signal transduction cascades.[1]

Hyperactivation of the EGFR family of receptors leading to uncontrolled cell proliferation is seen in a variety of cancers.[2] Activation of EGFR may be due to overexpression of the receptors (head and neck, lung, breast, bladder, prostate, and kidney tumors), mutations resulting in constitutive activation (brain tumors), or overexpression of the ligands (EGF or TGFα in pancreatic, prostate, lung, ovary, and colon cancers).[3,4] In contrast, activation of HER-2 occurs mainly via overexpression in breast, ovarian, lung, prostate, gastric, and oral cancers[2,5] leading to spontaneous homodimerization and activation of downstream signaling events in a ligand-independent manner. In particular, overexpression of HER-2 was found in 25−30% of breast cancer cases and has been correlated with poor prognosis in patients.[6]

Since deregulation of the EGFR and HER-2 tyrosine kinases has been associated with a variety of cancers, small-molecule inhibitors that could control the tyrosine phosphorylation event could be of therapeutic value as potential antitumor agents. Indeed, there are several ATP-competitive EGFR tyrosine kinase inhibitors currently in clinical trials for the treatment of cancer.[7,8] Among them, a 6,7-disubstituted 4-anilinoquinoline-3-

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carbonitrile **86** (EKB-569), developed by us,[9,10] is an orally active, irreversible inhibitor of the EGFR kinase. This compound carries a Michael acceptor at the 6-position, which forms a covalent bond to Cys 773 located in the ATP binding pocket of the EGFR enzyme. The water-solubilizing dimethylamino group at the end of the Michael acceptor serves as an intramolecular base catalyst operating via a cyclic five-membered ring mechanism to catalyze the Michael addition process.[9] This enhanced reactivity with the EGFR enzyme along with improved bioavailability resulted in **86** displaying potent antitumor activity in EGFR-dependent tumor models. However, since **86** shows less efficacy in HER-2 dependent tumor models, we continued with our efforts to develop irreversible inhibitors with improved activity toward HER-2 expressing tumors. These efforts are the subject of this communication.

EGFR and HER-2 share an 82% sequence identity in the kinase domains; the identity is even higher in the active site.[11] We constructed a homology model of HER-2 kinase for this study using the crystal structure of EGFR kinase in complex with erlotinib, a quinazoline-based inhibitor (also known as Tarceva, or CP-358774).[12] Since Cys 773 in EGFR is conserved as Cys 805 in HER-2, the development of an irreversible inhibitor of the latter kinase appeared to be feasible. The most notable difference in the ATP binding region between these kinases is a single amino acid residue, i.e., Ser 783 in HER-2 vs Cys 775 in EGFR. The high level of identity between the ATP binding regions of these two enzymes suggested that it would be difficult to design molecules that would be selective for one over the other. Designing an inhibitor that showed improved efficacy in HER-2 models (compared to **86**) appeared to be a more realistic undertaking. The binding models of our inhibitors indicated that the aniline portion fits into a long lipophilic pocket. Since this is the region of the binding pocket with the most difference between the two kinases and since there is room to introduce large lipophilic groups at the para position of the aniline, we explored various modifications to the 4-arylamino portion of the inhibitors in an empirical search to identify molecules with improved HER-2 activity. Interestingly, several other groups[13−15] have independently discovered, for quinazoline-based inhibitors, that large lipophilic substituents lead to improved HER-2 activity and, in some cases, improved dual HER-2/EGFR activity.[16,17] Here, we describe the synthesis and SAR of a series of 6,7-disubstituted 4-(arylamino)quinoline-3-carbonitriles modified with a variety of lipophilic substituents on the arylamino ring. Compared to our EGFR kinase inhibitor **86**, several of these compounds, as exemplified by **25o** (Figure 1), show improved activity toward HER-2 kinase while retaining good potency for EGFR kinase. Most importantly, this dual HER-2 and EGFR inhibitor, **25o**, also demonstrated improved in vivo efficacy in HER-2 dependent tumor models.

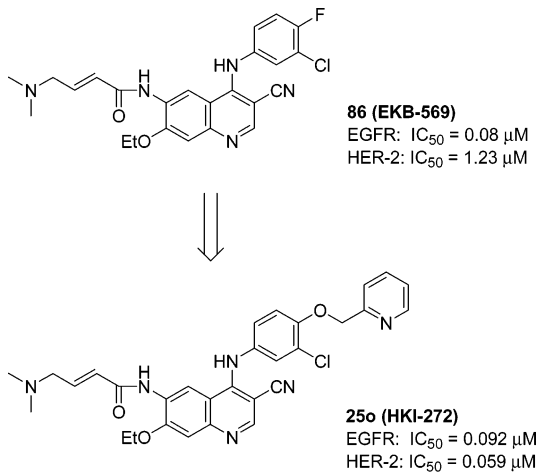


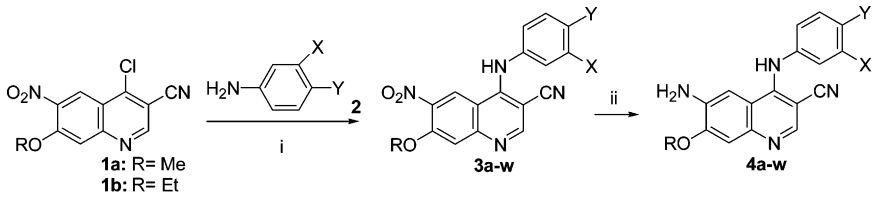
Figure 1.

Chemistry

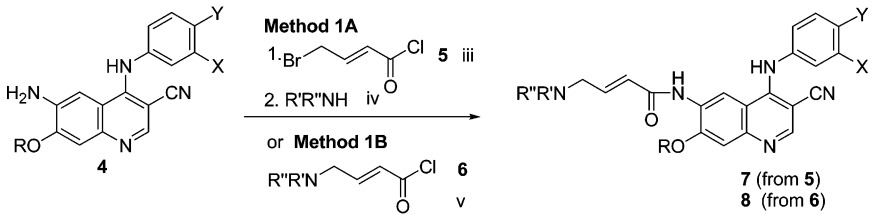
Most of the 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles (**7**, **8**, **21**, **25**) described here were prepared by one of the three methods shown in Schemes 1−3. In Scheme 1, 4-chloro-6-nitroquinoline-3-carbonitriles **1a**,**b**[9] were reacted with a substituted aniline **2** (including 5-amino-1-benzylindole and 5-amino-1-benzylindazole) and then reduced with iron and aqueous ammonium chloride to yield the corresponding 6-amino-4-anilinoquinoline-3-carbonitriles **4**, which were converted to the final products by two methods. In method 1A, the amines **4** were reacted with 4-bromocrotonyl chloride **5** and then the bromo group was displaced with secondary amines to yield the final compounds **7**. In method 1B, the final compounds **8** were prepared in one step by reacting the amines **4** with 4-(*N*,*N*-disubstituted amino)-crotonyl chlorides **6**. Both methods shown in Scheme 1 were used successfully in our early work[9] to prepare **86** and similar 4-anilinoquinoline-3-carbonitriles as inhibitors of EGFR kinase. Our original preparation of **1a**,**b** involved a high-temperature cyclization of a nitro-containing intermediate.[9] Although we never experienced any difficulty with this cyclization, we did have some concerns about performing the cyclization on a large scale. Therefore, we developed an alternative synthesis where the cyclization was carried out on an acetoamido intermediate **14** as shown in Scheme 2 (method 2). The commercially available 2-amino-5-nitrophenol **9** was protected as acetamide **10**. It was then ethylated and reduced to yield 4-acetamido-3-ethoxyaniline **12**, which upon condensation with ethyl (ethoxymethylene)cyano acetate **13** yielded the acetamido intermediate **14**. Cyclization of **14** was carried out in Dowtherm at 250−255 °C to yield 6-acetamido-4-hydroxyquinoline-3-carbonitrile **15**. Chlorination of **15** generated the 4-chloro intermediate **16**. The subsequent reactions are similar to those already described (Scheme 1) with the addition that the acetamido group of **18** was deacetylated to amine **19** before reacting with the desired crotonyl chloride **20** to yield the final compounds **21**.

A third synthetic method was developed where the 6-crotonamide side chain was first attached to the quinoline-3-carbonitrile ring, followed by introduction of the anilines at the C-4 position, as shown in Scheme 3 (method 3). 4-Chloro-6-nitro compound **1b** was reduced to the 4-chloro-6-amino compound **22**, which was then condensed with 4-(*N*,*N*-dimethylamino)crotonyl chloride to yield **23**. This was then converted to the final products **25** by heating with a variety of anilines **24** in methoxyethanol or 2-propanol using a catalytic amount of pyridine hydrochloride. This method was proved

Scheme 1. Methods 1A and 1B[a]



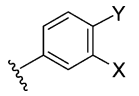
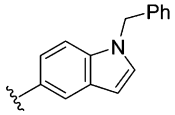
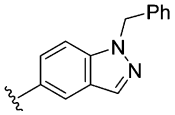
	R	X	Y
a	OMe	Cl	1-imidazolyl
b	OEt	Cl	*O*-(4-pyridinyl)
c	OMe	Cl	*S*-[2-(3-methylimidazolyl)]
d	OMe	Cl	*S*-(2-thiazolyl)
e	OEt	Cl	CH_2-(1-imidazolyl)
f	OEt	Cl	OCH_2-(3-F-phenyl)
g	OEt	Cl	OCH_2-phenyl
h	OEt	Cl	*N*-morpholinyl
i	OMe	Cl	*S*-(2-pyrimidyl)
j	OMe	Cl	*S*-[2-(5-phenylthiazolyl)]
k	OEt	Cl	*S*-[2-(4-phenylthiazolyl)]
l	OEt	Cl	CH_2-[2-(4-phenylthiazolyl)]
m	OMe	Cl	OCH_2-phenyl
n	OMe	H	OCH_2-phenyl
o	OEt	Cl	OCH_2-(2,4-di-F-phenyl)
p	OEt	Cl	OCH_2-(3-Cl-phenyl)
q	OEt	Cl	OCH_2-(2-OMe-phenyl)
r	OEt	Cl	OCH_2-[4-(OCH_2Ph)phenyl
s	OEt	Cl	OCH_2-(2-thienyl)
t	OEt	Cl	(1*R*)-2,3-dihydro-1*H*-inden-1-yloxy
u	OEt	Cl	(1*S*)-2,3-dihydro-1*H*-inden-1-yloxy
v	OEt	OCH_2-phenyl	H
w	OEt	Cl	N(Me)CH_2-phenyl



	R	X	Y	NR'R"
7a	OMe	Cl	1-imidazolyl	NMe_2
7b	OEt	Cl	*O*-(4-pyridinyl)	NMe_2
7c	OMe	Cl	*S*-[2-(3-methylimidazolyl)]	NMe_2
7d	OMe	Cl	*S*-(2-thiazolyl)	NMe_2
7e	OEt	Cl	CH_2-(1-imidazolyl)	NMe_2
7f	OEt	Cl	OCH_2-(3-F-phenyl)	NMe_2
7g	OEt	Cl	OCH_2-phenyl	N(Me)$(CH_2)_2$OMe
7h	OEt	Cl	OCH_2-phenyl	N(Me)$(CH_2)_2$OH
7i	OEt	Cl	OCH_2-phenyl	N(Me)-D-glucamine
7j	OEt	Cl	OCH_2-phenyl	N(*i*-Pr)$_2$
7k	OEt	Cl	OCH_2-phenyl	N[CH_2CH(OH)CH_3]$_2$
7l	OEt	Cl	OCH_2-phenyl	2-methoxymethyl-1-pyrrolidinyl
7m	OEt	Cl	OCH_2-phenyl	*N*-piperidinyl
7n	OEt	Cl	OCH_2-phenyl	4-OH-1-piperidinyl
7o	OEt	Cl	OCH_2-phenyl	4,4-dihydroxy-1-piperidinyl
7p	OEt	Cl	OCH_2-phenyl	2,6-dimethyl-1-piperidinyl
7q	OEt	Cl	OCH_2-phenyl	*N*-morpholinyl
7r	OEt	Cl	OCH_2-phenyl	*N*-thiomorpholinyl
7s	OEt	Cl	OCH_2-phenyl	*N*-(4-methylpiperazinyl)
7t	OEt	Cl	OCH_2-phenyl	*N*-azetidinyl
7u	OEt	Cl	OCH_2-phenyl	2-carbomethoxy-1-aziridinyl
7v	OEt	Cl	OCH_2-phenyl	1-aza-12-crown-4
8a	OEt	Cl	*N*-morpholinyl	NMe_2
8b	OEt	Cl	OCH_2-phenyl	NMe_2
8c	OMe	Cl	*S*-(2-pyrimidyl)	NMe_2

Scheme 1. (Continued)

	R	X	Y	NR'R"
8d	OMe	Cl	*S*-[2-(5-phenylthizaolyl)]	NMe_2
8e	OEt	Cl	*S*-[2-(4-phenylthizolyl)]	NMe_2
8f	OEt	Cl	CH_2-[2-(4-phenylthizaolyl)]	NMe_2
8i	OMe	Cl	OCH_2-phenyl	NMe_2
8j	OMe	H	OCH_2-phenyl	NMe_2
8k	OEt	Cl	OCH_2-(2,4-di-F-phenyl)	NMe_2
8l	OEt	Cl	OCH_2-(3-Cl-phenyl)	NMe_2
8m	OEt	Cl	OCH_2-(2-OMe-phenyl)	NMe_2
8n	OEt	Cl	OCH_2-[4-(OCH_2Ph)phenyl	NMe_2
8o	OEt	Cl	OCH_2-(2-thienyl)	NMe_2
8p	OEt	Cl	(1*R*)-2,3-dihydro-1*H*-inden-1-yloxy	NMe_2
8q	OEt	Cl	(1*S*)-2,3-dihydro-1*H*-inden-1-yloxy	NMe_2
8r	OEt	OCH_2-phenyl	H	NMe_2
8s	OEt	Cl	N(Me)CH_2-phenyl	NMe_2
8t	OEt	Cl	OCH_2-phenyl	*N*-pyrrolidinyl
8u	OEt	Cl	OCH_2-phenyl	4-imidazolyl

	R	NR'R"	
8g	Et	NMe_2	
8h	Me	NMe_2	

[a] (i) *i*-PrOH, pyridine hydrochloride, reflux; (ii) Fe, NH_4Cl, MeOH, H_2O, reflux; (iii) THF, Hunig base, 4-bromocrotonyl chloride, 0 °C; (iv) DMF, NaI, R′R′NH, room temperature; (v) *N*-methyl-2-pyrrolidinone or DMF, from 0 °C to room temperature.

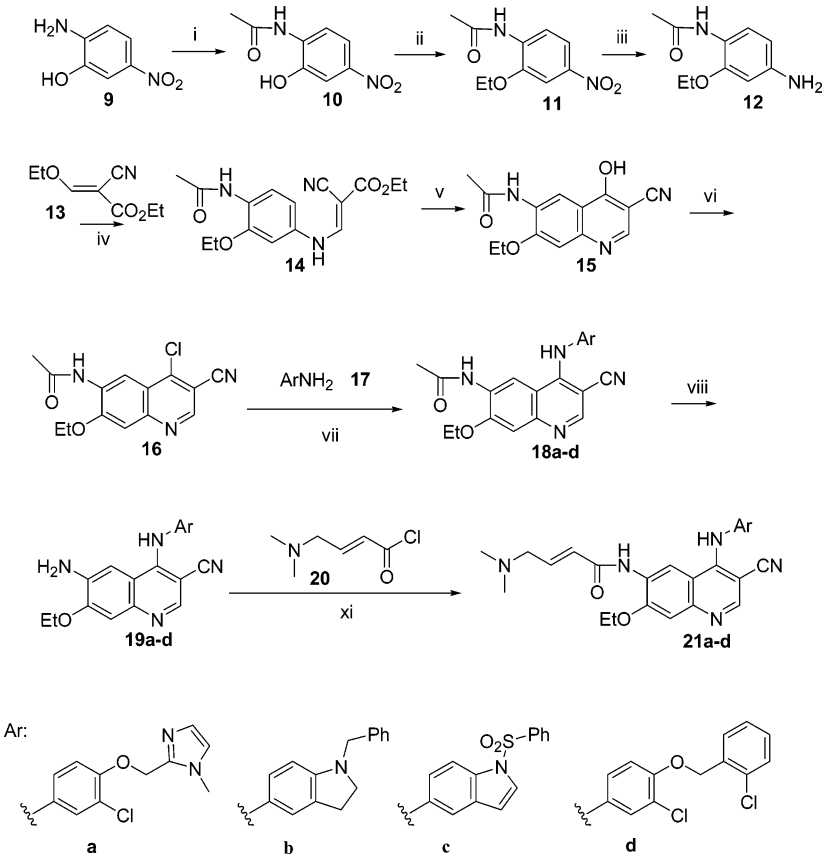
useful when optimizing the 4-anilino groups for HER-2 activity while keeping the 6-crotonamide side chain constant.

For some compounds (**31**, **32**, **36**−**38**) where the 4-aniline ring bears nitrogen-containing para substituents, the synthetic pathways are shown in Schemes 4 and 5. The amino group of **26** was first protected with BOC anhydride, the nitro group was reduced to yield the aniline **28**, and this was condensed with chloride **23** followed by deprotection of the BOC group to yield **29**. Reductive amination of the amino group of **29** with aldehydes **30** using sodium triacetoxyborohydride in acetic acid gave **31** and **32**. An alternative approach was used to prepare the 7-methoxy intermediate **35** as shown in Scheme 5. The 4-chloro compound **1a** was first condensed with aniline **28**, then the nitro group was reduced followed by the reaction of **34** with 4-(*N,N*-dimethylamino)crotonyl chloride in a manner similar to that shown in Scheme 1 (method 1B). After deprotection of the BOC group with TFA, the key intermediate **35** was obtained. Condensing **35** with acid anhydride, acid chloride, or phenyl isocyanate yielded amides **36** and **37** or urea **38**.

The preparations of the substituted anilines that were needed to prepare some of the inhibitors are shown in Schemes 6 and 7. 3-Chloro-4-fluoronitrobenzene was reacted with 5-phenylthiazole-2-thiol under basic conditions to yield **39**, which was then reduced with iron and acetic acid in methanol to yield the aniline **40**. A similar synthetic sequence was used in the reaction of 3-chloro-4-fluoronitrobenzene with phenols, alcohols, and amines. 4-(1*H*-Imidazole-1-ylmethyl)-3-chloroaniline **42** was prepared by alkylation of imidazole with 2-chloro-4-nitrobenzyl bromide to yield **41**, followed by reduction of the nitro group. The 4-benzyoxyaniline **44** was readily prepared by reacting phenol **43** with benzyl bromide under basic conditions, followed by reduction of the nitro group as shown. The synthesis of 3-chloro-4-(4-phenylthiazol-2-ylmethyl)phenylamine, **51**, where the thiazole group is attached via a carbon atom instead of a heteroatom, is shown in Scheme 7. The toluene derivative **45** was heated with *tert*-butoxy-*N,N,N′,N′*-tetramethylmethanediamine, **46**, to yield the enamine **47**,[18] which was then converted to the phenylacetonitrile **48** by reacting with hydroxylamine-*O*-sulfonic acid in warm water.[19] Heating the nitrile **48** with H_2S in triethylamine and DMF in a steel bomb gave the thioacetamide **49**, which was then condensed with phenacyl bromide **50** to yield the desired aniline **51**. Scheme 8 shows the synthesis of several bicyclic anilines. 5-Amino-1-benzylindole **54** was prepared by benzylation of the 5-nitro-1*H*-indole **52**, followed by reduction of the nitro group to the amine. 5-Amino-1-benzylindazole **55** and 5-amino-2,3-dihydro-1-benzylindole **57** were prepared in a similar manner. 5-Amino-1-phenylsulfonylindole **59** was synthesized similarly except that phenylsulfonyl chloride was used.

Preparations of some representative Michael acceptor side chains are shown in Schemes 9 and 10. *tert*-Butyl 4-bromocrotonamide **60**[20] was converted into the corresponding iodide **61** by refluxing with sodium iodide in acetone. The iodide **61** was reacted with a variety of secondary amines such as pyrrolidine at 0 °C to yield amino ester **62**. After removal of the *tert*-butyl group

Scheme 2. Method 2[a]



[a] (i) Ac_2O, HOAc, 60 °C; (ii) EtBr, DMF, K_2CO_3, 60 °C; (iii) H_2, Pd/C, THF; (iv) toluene, 90 °C; (v) Dowtherm, 250 °C; (vi) $POCl_3$, diglyme, 100 °C; (vii) pyridine hydrochloride, *i*-PrOH or methoxyethanol, reflux; (viii) aqueous HCl, reflux; (xi) CH_3CN, *N*-methyl-2-pyrrolidinone or DMF, from 0 °C to room temperature.

under acidic conditions, a suspension of amino acid HCl salt **63** in CH_3CN containing a catalytic amount of *N*-methyl-2-pyrrolidinone (or DMF) was heated with oxalyl chloride to afford the acid chloride **64** as a hydrochloride salt, which was used immediately in subsequent reactions. 4-(1*H*-Imidazol-4-yl)-but-2-enoic acid hydrochloride, **67**, was prepared by reacting 1-trityl-1*H*-imidazole-4-acetate **65**[21] with triethyl phosphonoacetate and *n*-BuLi in THF at −78 °C and then with DIBAL[22] to yield **66** (Scheme 10). Ester hydrolysis and removal of the trityl group from **66** were followed by acid chloride formation to give **68**. 3-(2-Dimethylaminophenyl)acrylic acid **70** was prepared by heating 2-(dimethylamino)benzaldehyde **69**[23] in pyridine with malonic acid using a catalytic amount of piperidine. Acid **70** was then converted to acid chloride HCl salt **71** using oxalyl chloride. Acid chlorides **73** and **74** were prepared in a similar manner (Scheme 11).

Compounds with other types of Michael acceptors (**82**−**85**) were prepared as shown in Scheme 12. The 6-amino derivative **72** was treated with 2-chloro-1-ethanesulfonyl chloride **78** in triethylamine at 0 °C, resulting in the vinylsulfonamide **82**. Preparation of acid chlorides **79** and **80** were reported earlier;[24] these were reacted with **72** to yield **83** and **84**, respectively. Finally, commercially available 2-(bromomethyl)acrylic acid **81** was reacted with **72** to afford **85**.

Molecular Modeling

A homology model for the catalytic domain of HER-2 kinase was built as follows. The crystal structure of the highly homologous EGFR kinase in complex with erlotinib[12] was used as the template. Appropriate side chain substitutions were made, a large sphere of water molecules were added, and the whole structure was minimized using Quanta/CHARMm.[25]

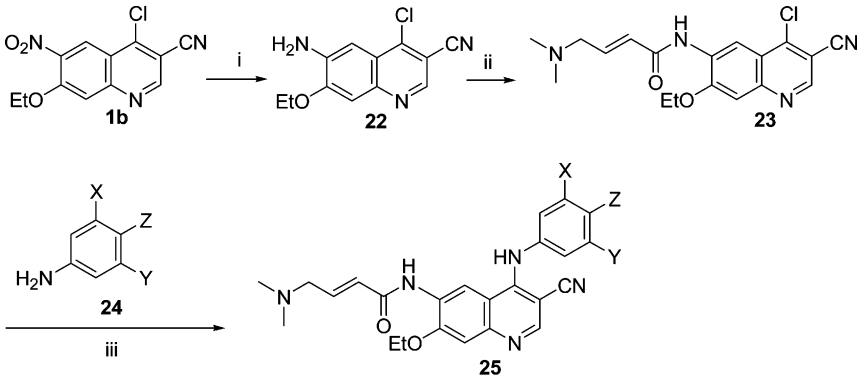
By use of this model, ligands were hand-docked in accordance with our previous studies of compounds in the quinazoline/cyanoquinoline class,[9,10,26] and the EGFR−erlotinib complex.[12] The complex was again optimized using Quanta/CHARMm. Because EGFR and HER-2 are closely related in sequence, the construction of the homology model presented no significant challenges.

In the final model of the HER-2−**25o** complex (see Figure 2), the N1 atom of the quinoline is hydrogen-bonded to the backbone NH of Met 801 at a distance of 3.32 Å. The cyano nitrogen is 2.76 Å from the OH of Ser 783. The chloroaniline portion of the inhibitor is surrounded by residues Thr 798, Lys 753, Leu 133, Thr 143, and Asp 144, while the pyridylmethoxy group lies in a pocket surrounded by Val 773, Met 774, Arg 784, Ser 783, Val 777, Phe 864, and Thr 862.

The sulfur of Cys 805 is 3.43 Å from the reactive Michael acceptor carbon. The dimethylamino group, which we have shown earlier[9] can function as an intramolecular catalyst for Michael additions, is also close to the sulfhydryl hydrogen of Cys 805.

A paper describing a study of the binding modes of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles to the closely related EGFR kinase appeared recently.[27] In it, the authors contend that they were unable to

Scheme 3. Method 3[a]



	X	Y	Z
a	H	Cl	O-phenyl
b	H	H	OCH_2-phenyl
c	H	Cl	$O(CH_2)_2$-phenyl
d	Cl	Cl	OCH_2-phenyl
e	H	CN	OCH_2-phenyl
f	H	Cl	OCH_2-(2-F-phenyl)
g	H	Cl	OCH_2-(4-F-phenyl)
h	Cl	Cl	OCH_2-(3-F-phenyl)
i	H	Cl	OCH_2-(3,5-di-F-phenyl)
j	H	Cl	OCH_2-(4-Cl-phenyl)
k	H	Cl	OCH_2-(3-CN-phenyl)
l	H	Cl	$O(CH_2)_4CH_3$
m	H	Cl	OCH_2-(3-Me-phenyl)
n	H	Cl	OCH_2-cyclohexyl
o	H	Cl	OCH_2-(2-pyridinyl) (HKI-272)
p	H	Cl	OCH_2-(3-pyridinyl)
q	H	Cl	OCH_2-(2-furyl)
r	H	Cl	OCH_2-(2-benzothiazolyl)
s	H	Cl	*O*-(4-quinolinyl)
t	H	Cl	OCH_2-(1-naphthyl)
u	H	Cl	OCH_2-(2-naphthyl)
v	H	Cl	[(1*R*)-1-phenylethyl]oxy
w	H	Cl	[(1*S*)-1-phenylethyl]oxy

[a] (i) Fe, HOAc, NaOAc, MeOH, reflux; (ii) $Me_2NCH_2CH{=}CHCOCl$ in CH_3CN, *N*-methyl-2-pyrrolidinone or DMF, from 0 °C to room temperature; (iii) pyridine hydrochloride, methoxyethanol, or *i*-PrOH, reflux.

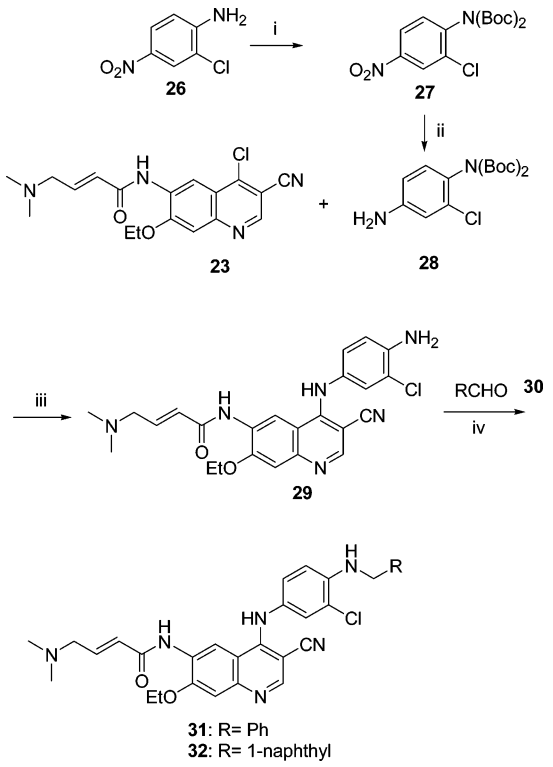
reproduce a binding mode such as ours where the headpiece is buried deep into the ATP binding pocket and the Michael acceptor is positioned suitably to interact with Cys 773 (analogous to Cys 805 of HER-2 kinase). They reported a distance of 5.97 Å between the β-carbon and the sulfur of Cys 773 in their model. We point out that the ligand and the protein have considerable flexibility even when complexed. Thermal motion of the protein and ligand atoms should, in our opinion, be adequate to bring the β-carbon and the sulfur of Cys 773 close enough to form a covalent interaction. A close examination of the EGFR−erlotinib crystal structure[12] leads us to the same conclusion.

Results and Discussion

Kinase and Cellular Inhibitory Activities. The compounds shown in Tables 1−5 were evaluated for their ability to inhibit HER-2 and EGFR kinases. While some other researchers may determine IC_{50} values of inhibitors in soluble formats using the entire HER-2 and EGFR enzymes along with exogenous peptide substrates, we use a solid-phase ELISA-based assay consisting of the purified cytoplasmic domain of HER-2 (amino acids 676−1255) or EGFR (amino acids 645−1186). Concentrations of compounds to inhibit autophosphorylation of these proteins by 50% were measured to generate the IC_{50} values. As we indicated in our earlier publications, the IC_{50} values generated by our method are routinely higher than the IC_{50} values produced by other researchers using the full-length enzymes and exogenous peptide substrates, but the relative potencies remain consistent.

In an earlier report, we showed that **86** functions as an irreversible inhibitor of EGFR kinase.[28] We will show herein that compounds, as exemplified by **25o**, bind irreversibly to HER-2 kinase. As we have cautioned in our earlier publications,[9,24] care should be taken in interpreting the IC_{50} values of compounds that function as irreversible inhibitors. The IC_{50} values for such inhibitors should depend on the extent to which the covalent interaction has occurred. In addition, we have suggested, and it has been recapitulated by others,[27] that the IC_{50} values could arise from two components, one reflecting reversible binding and another reflecting

Scheme 4[a]



[a] (i) $(Boc)_2O$, DMAP, THF, 0 °C to room temperature; (ii) Fe, HOAc, MeOH, reflux; (iii) pyridine hydrochloride, methoxyethanol, reflux, then CH_2Cl_2, TFA, from 0 to 25 °C; (iv) $NaBH(OAc)_3$, dichloroethane, HOAc, room temperature.

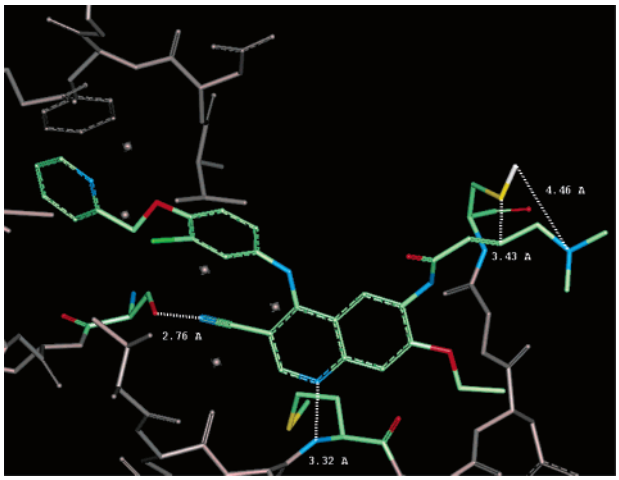


Figure 2. Model of the HER-2−**25o** complex. The H-bond between the quinoline N1 and Met 801 is shown. Also shown are distances between the sulfur of Cys 805 and the Michael acceptor β-carbon, and the sulfhydryl hydrogen and the nitrogen of the dimethylamino group, the suggested intramolecular catalyst.

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 because of 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 with varying

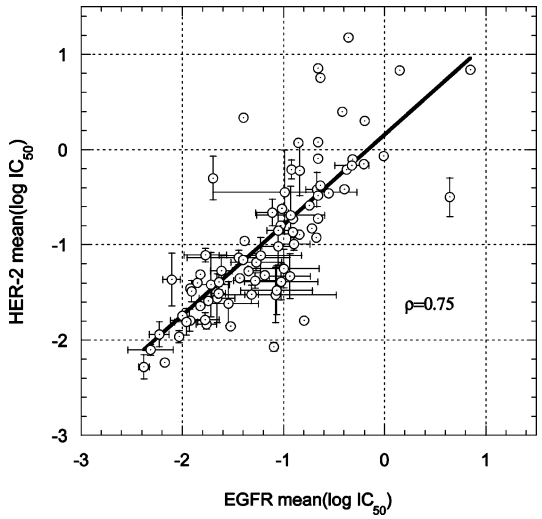


Figure 3. Scatter plot comparing the activities of compounds against the EGFR and HER-2 kinase enzymes. Note that mean values of log IC_{50} are plotted. The error bars (horizontal for EGFR, vertical for HER-2) represent the standard error of the respective mean values. Note that the number of observations for each compound varies from 1 to 30. The single-point observations show no error bars. The least-squares regression line is also shown.

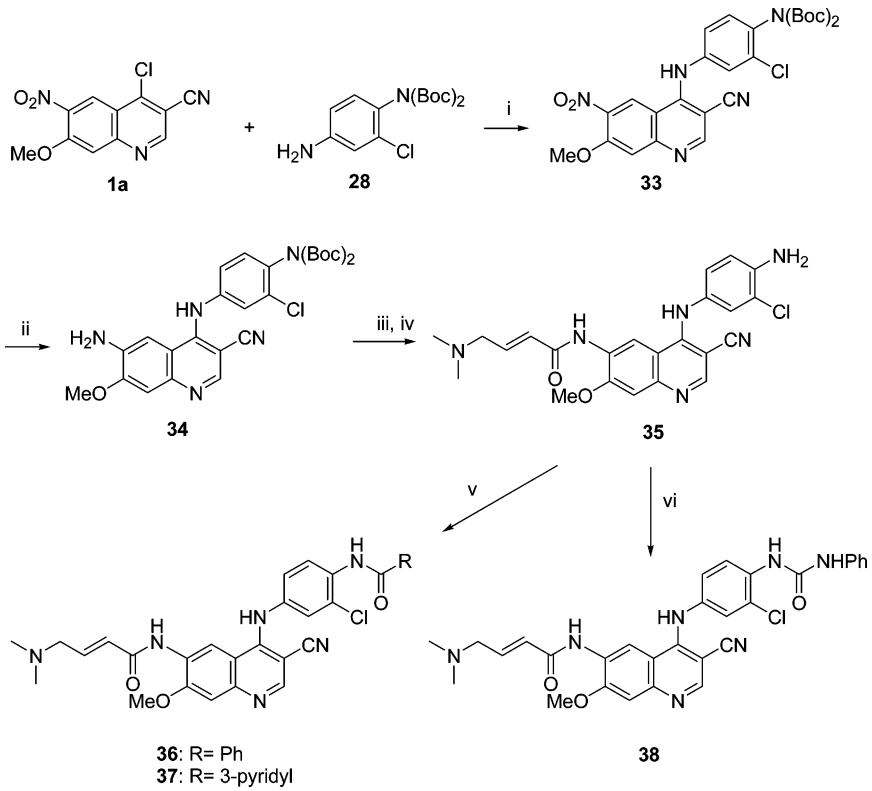
levels of expression of HER-2 and/or EGFR (Tables 1−5). Three human carcinoma cell lines were used: A431 (epidermoid), which overexpresses EGFR; SKBR3 (breast), which overexpresses HER-2 and, to a lesser extent, EGFR; SW620 (colon), which serves as a control line not expressing either EGFR or HER-2 to a significant extent.

To get a global view of these data (Tables 1−5), a matrix of correlation coefficients was constructed and is shown in Table 6. It is evident that there is a reasonable correlation between the EGFR and HER-2 inhibitory activities. This is shown in detail in the scatter plot of the HER-2 and EGFR activities (Figure 3). This correlation is expected 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 these growth factor receptors. The correlation between the A431 and SKBR3 cell lines data is significantly higher than the correlation between either of these data and the SW620 cell line results.

On the other hand, we see a low correlation between the kinase 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 permeability, play a role here, this might be expected. Likewise, the kinase data do not correlate well with the SW620 cell activities.

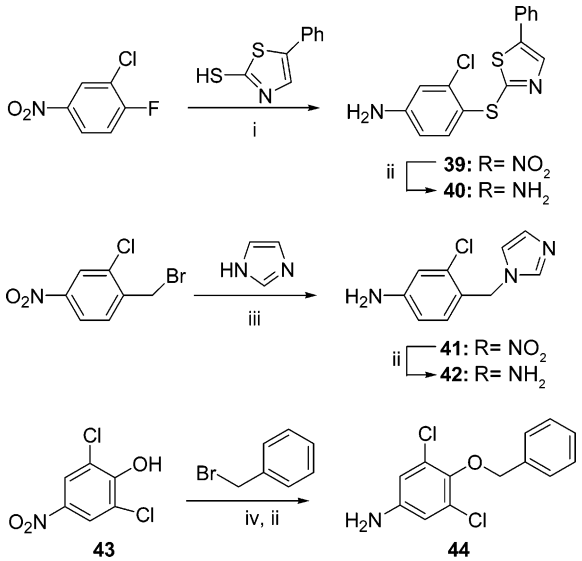
As mentioned in the Introduction, we adopted the same chemical scaffold used with our irreversible EGFR inhibitor **86** and focused our synthetic efforts on introducing large lipophilic groups at the para position of the 3-chloroaniline ring because this portion of the molecule, when bound, resides in the region where the enzymes are most different. Since our efforts were directed

Scheme 5[a]



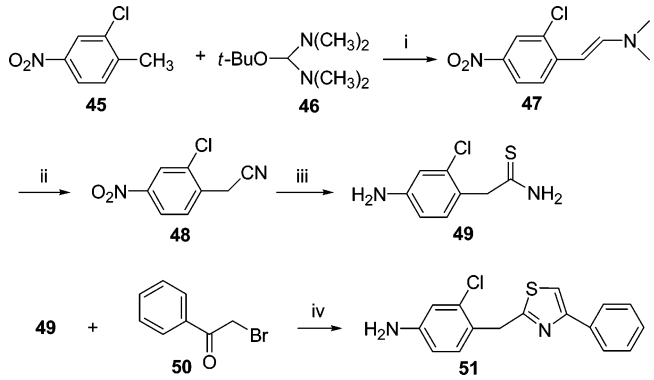
[a] (i) *i*-PrOH, reflux; (ii) Fe, HOAc, MeOH, reflux; (iii) $Me_2NCH_2CH{=}CHCOCl$ in CH_3CN, *N*-methyl-2-pyrrolidinone or DMF, from 0 °C to room temperature; (iv) CH_2Cl_2, TFA; (v) $(PhCO)_2O$, DMA, 60 °C for **36**, nicotinyl chloride hydrochloride, $(i\text{-Pr})_2NEt$, DMA, 25 °C for **37**; (vi) Ph-N=C=O, DMA, 25 °C.

Scheme 6[a]



[a] (i) NaH, DMF, room temperature, or NaOMe, DMF; (ii) Fe, NH_4Cl, MeOH, H_2O, reflux, or Fe, HOAc, MeOH, reflux; (iii) THF, reflux; (iv) Cs_2CO_3, DMF, 25 °C.

Scheme 7[a]

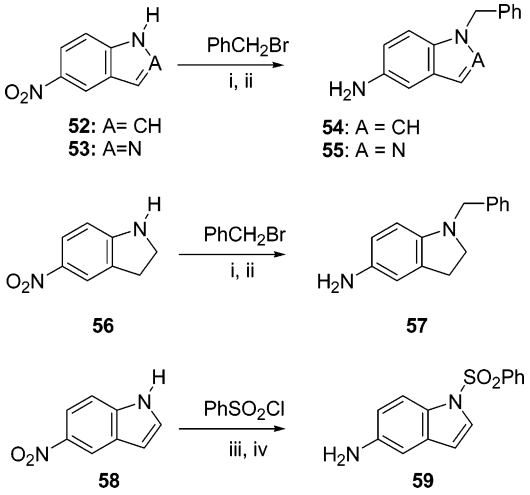


[a] (i) Heat and distill at 160 °C; (ii) H_2NOSO_3H, hot H_2O, 55 °C; (iii) Et_3N, DMF, H_2S, heat at 80 °C in a steel bomb; (iv) EtOH, reflux.

toward finding compounds with improved HER-2 activity relative to **86** (EKB-569), we discuss the data using this compound as a reference.

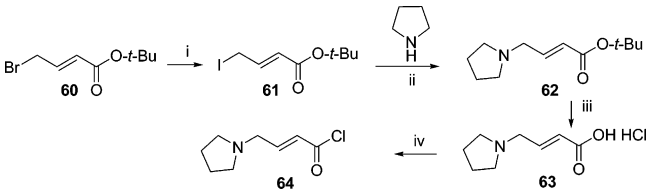
The compounds listed in Table 1 represent our initial exploration into the effects of placing large lipophilic groups at the para position of the 4-anilino substituent. It is evident that **7d**, bearing a 2-thiazolsulfanyl substituent, is 15-fold more potent than **86** in inhibiting the HER-2 kinase while retaining similar potency as **86** for EGFR kinase. The compound also demonstrated better activity for the HER-2 overexpressing cell line SKBR3. To further increase lipophilicity and steric bulk, a phenyl group was introduced at the C-5 (**8d**) and C-4 (**8e**) positions of the thiazole ring. While **8d** is a significantly less potent inhibitor of HER-2 kinase compared to **7d**, the regioisomer **8e** is 6-fold more potent than **7d** in the HER-2 assay. Also, when the thioether linkage of **8e** was replaced with a methylene bridge, the resulting compound **8f** remained as potent as **8e**. In addition, other compounds with large lipophilic substituents were prepared showing enhanced activity toward HER-2 kinase compared to **86**. It is evident that for HER-2 inhibition, the benzyloxy compound **8b** and the phenoxy compound **25a** are significantly more potent

Scheme 8[a]



[a] (i) K_2CO_3, DMF, heat; (ii) Fe, HOAc, MeOH, H_2O, reflux; (iii) NaH,benzene; (iv) Raney Ni, hydrazine, EtOH.

Scheme 9[a]

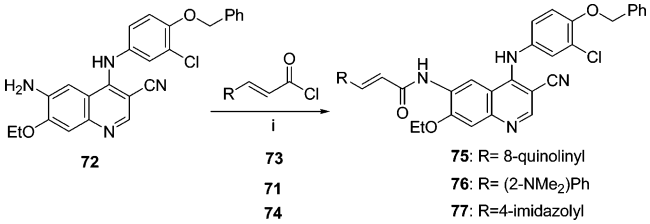


[a] (i) NaI, acetone, reflux; (ii) THF, 0 °C; (iii) 1 N HCl, reflux; (iv) CH_3CN, $(COCl)_2$, *N*-methyl-2-pyrrolidinone or DMF (cat.), 55 °C.

than **86** (16-fold and 7-fold, respectively) while the corresponding 4-(pyridinyl)oxy compound **7b** is significantly less potent. Furthermore, **8b** has about the same potency as **86** against the EGFR kinase. These results are similar to those found by other researchers in the quinazoline-based series, where enhancement of HER-2 activity was observed for compounds bearing bulky substituents on the 4-aniline ring.[14,16,17]

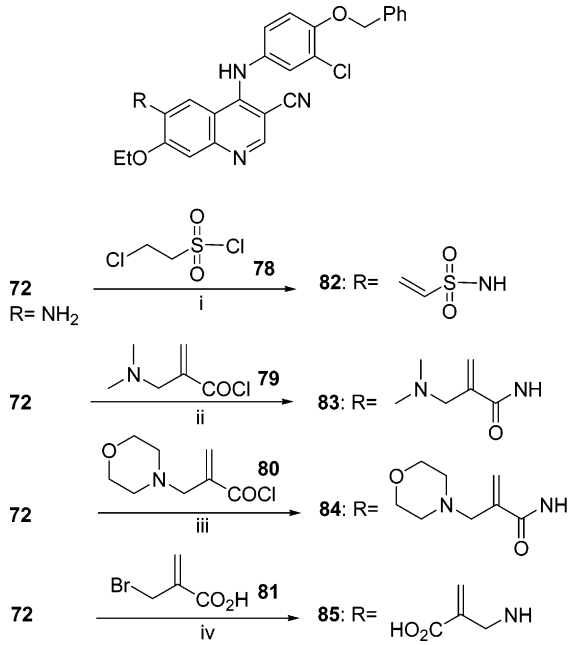
We also looked at several compounds that have bicyclic aniline substituents such as **21b**, **21c**, **8g,** and **8h** in Table 2. These compounds are significantly more potent than **86** in inhibiting HER-2 kinase, and they show good activity in inhibiting the growth of the SKBR3 cell line.

Scheme 11[a]



[a] (i) *N*-Methyl-2-pyrrolidinone, from 0 °C to room temperature.

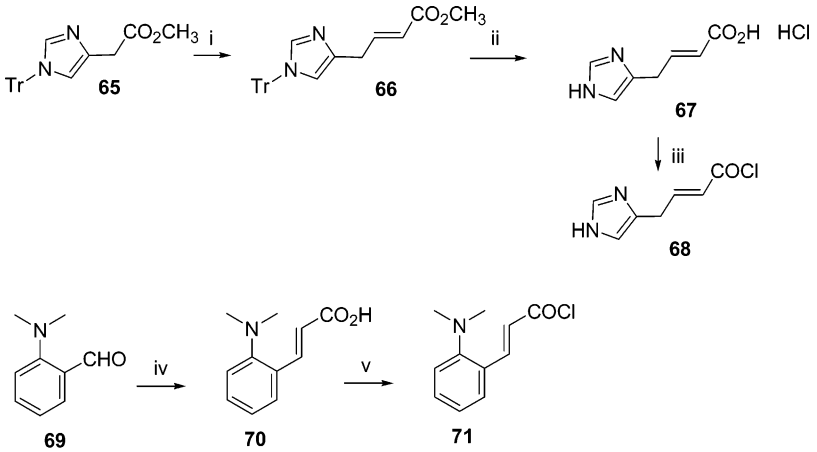
Scheme 12[a]



[a] (i) THF, Et_3N, 0 °C; (ii) *N*-methyl-2-pyrrolidinone, from −15 °C to room temperature; (iii) *N*-methyl-2-pyrrolidinone, from 0 °C to room temperature; (iv) CH_3CN, Hunig's base, reflux.

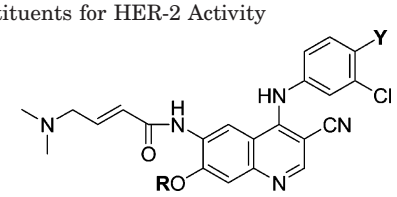
Throughout this work, we used either a methoxy or ethoxy substituent at the 7-position. There is no clear advantage of either substituent with respect to potency (for example, compare **8b** with **8i** and compare **25a** with **8j** in Table 3). However, from our early work, we found that a number of the inhibitors that have a 7-methoxy substituent showed a very weak positive response in an Ames test after microsomal incubation while the cor-

Scheme 10[a]



[a] (i) Triethyl phosphonoacetate, *n*-BuLi, THF, then DIBAL, CH_2Cl_2, −78 °C to room temperature; (ii) MeOH, NaOH, then HCl; (iii) CH_2Cl_2, $(COCl)_2$, cat. DMF, reflux; (iv) malonic acid, pyridine, piperidine (cat.), 110 °C; (v) CH_2Cl_2, $(COCl)_2$, DMF (cat.), reflux.

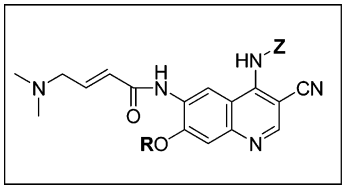
Table 1. Initial Exploration of the Para Substituents for HER-2 Activity



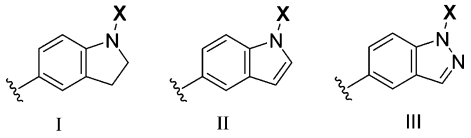
compd	R	Y	synthetic method	kinase assays IC_{50} (μM) HER-2[a]	kinase assays IC_{50} (μM) EGFR[b]	cell proliferation assays IC_{50} (μM) A431[c]	cell proliferation assays IC_{50} (μM) SKBR3[c]	cell proliferation assays IC_{50} (μM) SW620[c]
7a	Me	1-imidazolyl	1A	0.80	0.77	1.2	0.40	2.7
7b	Et	*O*-(4-pyridinyl)	1A	8.1	0.65	6.0	2.8	>9
7c	Me	*S*-[2-(3-methylimidazolyl)]	1A	0.155	0.041	0.132	0.0013	1.093
7d	Me	*S*-(2-thiazolyl)	1A	0.083	0.042	0.516	0.0015	1.592
7e	Et	CH_2-(1-imidazolyl)	1A	1.055	0.519	2.839	0.1094	5.48
8a	Et	4-morpholinyl	1B	2.6	12.7	0.43	1.2	2.1
8b	Et	OCH_2Ph	1B	0.076	0.053	0.108	0.0027	0.486
8c	Me	*S*-(2-pyrimidyl)	1B	0.348	0.403	0.209	0.0156	1.300
8d	Me	*S*-[2-(5-phenylthiazolyl)]	1B	1.007	0.032	0.574	0.0462	0.961
8e	Et	*S*-[2-(4-phenylthiazolyl)]	1B	0.013	0.125	0.374	0.0094	0.842
8f	Me	CH_2-[2-(4-phenylthiazolyl)]	1B	0.074	0.214	0.082	0.0049	0.328
21a	Et	*O*-CH_2-[2-(3-methylimidazolyl)]	2	0.625	0.500	0.129	0.0021	0.993
25a	Et	*O*-Ph	3	0.184	0.445	0.052	0.0039	0.683
86	Et	F		1.23	0.08	0.08	0.01	0.68

[a] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50% as determined from the dose–response curve. [b] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50% as determined from the dose–response curve. [c] Dose–response curves were determined at five concentrations. The IC_{50} (μM) values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

Table 2. 2,3-Dihydro-1*H*-indole, 1-*H*-indole, and 1*H*-Indazole Inhibitors



Z:



I II III

compd	series	R	X	synthetic method	kinase assays IC_{50} (μM) HER-2[a]	kinase assays IC_{50} (μM) EGFR[b]	cell proliferation assays IC_{50} (μM) A431[c]	cell proliferation assays IC_{50} (μM) SKBR3[c]	cell proliferation assays IC_{50} (μM) SW620[c]
8g	II	Et	CH_2Ph	1B	0.015	0.10	0.084	0.0014	0.74
8h	III	Me	CH_2Ph	1B	0.075	0.13	0.1	0.0034	0.87
21b	I	Et	CH_2Ph	2	0.23	0.34	0.23	0.028	2.1
21c	II	Et	SO_2Ph	2	0.025	0.082	0.17	0.015	1.1

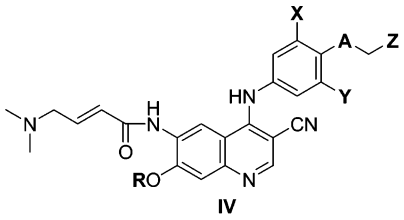
[a] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50% as determined from the dose–response curve. [b] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50% as determined from the dose–response curve. [c] Dose–response curves were determined at five concentrations. The IC_{50} (μM) values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

responding ethoxy derivatives did not. Because of this observation, the majority of the compounds prepared possessed the 7-ethoxy substituent.

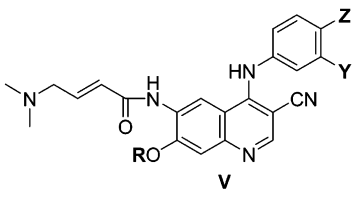
Preliminary in vivo experiments indicated that of the compounds we initially evaluated (Tables 1 and 2), the benzyl-substituted derivative **8b** looked the most promising for further modifications. Replacement of the benzyloxy group of **8b** with the slightly longer phenylethoxy group results in a compound, **25c**, that is slightly less potent in both kinase and cell proliferation assays (Table 3). By comparision of the activity of **25a**, **8b**, and **25c**, it is clear that the chain length of the para substituent contributes to potency in inhibiting HER-2 kinase in the order of benzyloxy > phenoxy > phenylethoxy. The position of the benzyloxy substituent is also important for activity, with a preference for the 4-position of the 3-chloroaniline ring. Switching the benzyloxy substitutent from the 4- to the 3-position by replacing the 3-chloro group gives **8r**, which is clearly an inferior inhibitor of both HER-2 and EGFR kinases.

It is interesting to note the difference in the kinase activities between the pair of isomers where a methyl group was introduced at the methylene bridge of the benzyloxy group. While the *R* isomer (**25v**) showed potent kinase activity, the corresponding *S* isomer (**25w**) was 340-fold less potent for the HER-2 enzyme, suggesting a space requirement in the lipophilic pocket. Conformationally restricting the benzyloxy group by using 2,3-dihydro-1*H*-inden-1-yloxy substituents having both *R* and *S* isomer configurations (**8p** and **8q**, respectively) results in inhibitors with poor HER-2 kinase and to a lesser extent for EGFR activity relative to **8b**. Additional analogues of **8b** were prepared wherein the benzyloxy group was replaced with benzylamine (**31**), *N*-methylbenzylamine (**8s**), and 1-naphthylmethylamine (**32**). The benzylamine analogue **31** is as potent as **8b**, whereas **8s** shows much reduced activity. Poor activity was also observed for compounds where the benzylamine group in **31** was replaced with benzamide (**36**), nicotinamide (**37**), and phenylurea (**38**).

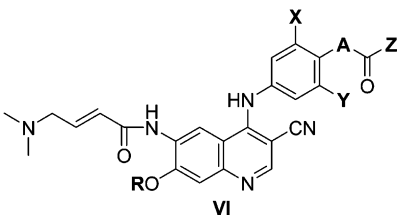
Table 3. Optimization of the Aniline Substituents for HER-2 Activity



IV



V



VI

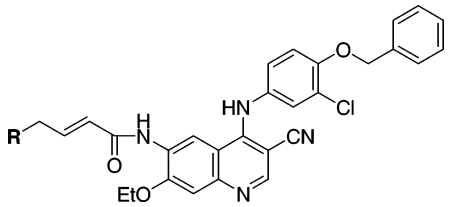
compd	series	R	X	Y	A	Z	synthetic method	kinase assays IC_{50} (μM) HER-2[a]	kinase assays IC_{50} (μM) EGFR[b]	cell proliferation assays IC_{50} (μM) A431[c]	cell proliferation assays IC_{50} (μM) SKBR3[c]	cell proliferation assays IC_{50} (μM) SW620[c]
7f	IV	Et	H	Cl	O	3-F-Ph	1A	0.033	0.034	0.120	0.0025	0.511
8b	IV	Et	H	Cl	O	Ph	1B	0.076	0.053	0.108	0.0027	0.486
8i	IV	Me	H	Cl	O	Ph	1B	0.039	0.024	0.083	0.0038	1.120
8j	IV	Me	H	H	O	Ph	1B	0.363	0.185	0.108	0.0098	1.019
8k	IV	Et	H	Cl	O	2,4-di-F-Ph	1B	0.081	0.110	0.211	0.0033	0.549
8l	IV	Et	H	Cl	O	3-Cl-Ph	1B	0.008	0.007	0.148	0.0012	0.965
8m	IV	Et	H	Cl	O	2-OMe-Ph	1B	0.044	0.031	0.307	0.0022	0.580
8n	IV	Et	H	Cl	O	4-(OCH_2Ph)Ph	1B	3.775	0.574	0.680	0.0800	1.404
8o	IV	Et	H	Cl	O	2-thienyl	1B	0.131	0.277	0.077	0.0033	0.307
8p	V	Et		Cl		(1*R*)-2,3-dihydro-1*H*-inden-1-yloxy	1B	2.1	0.38	0.069	0.0023	0.29
8q	V	Et		Cl		(1*S*)-2,3-dihydro-1*H*-inden-1-yloxy	1B	9.8	0.40	0.11	0.0063	0.3
8r	V	Et		OCH_2Ph		H	1B	13.2	13	0.53	0.04	1.43
8s	IV	Et	H	Cl	NMe	Ph	1B	2.1	0.25	0.37	0.034	-
21d	IV	Et	H	Cl	O	2-Cl-Ph	2	0.010	0.011	0.120	0.0029	0.948
25a	V	Et		Cl		OPh	3	0.184	0.445	0.052	0.0039	0.683
25b	IV	Et	H	H	O	Ph	3	0.498	0.345	0.136	0.0016	0.924
25c	IV	Et	H	Cl	O	CH_2Ph	3	0.281	0.165	0.254	0.0319	0.500
25d	IV	Et	Cl	Cl	O	Ph	3	0.406	0.163	0.423	0.0110	1.219
25e	IV	Et	H	CN	O	Ph	3	1.134	0.768	0.342	0.0059	0.540
25f	IV	Et	H	Cl	O	2-F-Ph	3	0.732	0.401	0.285	0.0028	1.472
25g	IV	Et	H	Cl	O	4-F-Ph	3	0.078	0.064	0.102	0.0082	0.584
25h	IV	Et	Cl	Cl	O	3-F-Ph	3	1.331	0.362	0.970	0.0378	1.200
25i	IV	Et	H	Cl	O	3,5-di-F-Ph	3	0.388	0.140	0.262	0.0033	1.073
25j	IV	Et	H	Cl	O	4-Cl-Ph	3	0.024	0.051	0.069	0.0093	0.593
25k	IV	Et	H	Cl	O	3-CN-Ph	3	0.161	0.195	0.071	0.0019	2.220
25l	IV	Et	H	Cl	O	$(CH_2)_3CH_3$	3	1.477	0.894	0.114	0.0078	0.410
25m	IV	Et	H	Cl	O	3-Me-Ph	3	0.132	0.111	0.052	0.0127	0.357
25n	IV	Et	H	Cl	O	cyclohexyl	3	0.028	0.285	0.151	0.0011	0.551
25o	IV	Et	H	Cl	O	2-pyridinyl (HKI-272)	3	0.059	0.092	0.086	0.0018	0.730
25p	IV	Et	H	Cl	O	3-pyridinyl	3	0.269	0.341	0.056	0.0004	0.197
25q	IV	Et	H	Cl	O	2-furyl	3	0.062	0.023	0.089	0.0037	0.366
25r	IV	Et	H	Cl	O	2-benzothiazolyl	3	0.064	0.188	0.864	0.0110	2.147
25s	IV	Et	H	Cl	O	4-quinolinyl	3	1.184	0.238	0.708	0.0631	3.261
25t	IV	Et	H	Cl	O	1-naphthyl	3	0.020	0.010	0.564	0.0127	0.973
25u	IV	Et	H	Cl	O	2-naphthyl	3	0.018	0.015	0.569	0.0280	0.775
25v	V	Et		Cl		[(1*R*)-1-phenylethyl]oxy	3	0.076	0.10	0.053	0.0013	0.14
25w	V	Et		Cl		[(1*S*)-1-phenylethyl]oxy	3	26	0.77	0.15	0.013	0.193.8
31	IV	Et	H	Cl	NH	Ph		0.103	0.045	0.048	0.0020	0.578
32	IV	Et	H	Cl	NH	1-naphthyl		0.083	0.034	0.17	0.023	0.93
36	VI	Me	H	Cl	NH	Ph		13	0.40	0.81	>5	0.62
37	VI	Me	H	Cl	NH	3-pyridyl		3.9	0.072	0.1	0.086	2.7
38	VI	Me	H	Cl	NH	NHPh		1.1	0.21	2.6	0.50	>5

[a] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50% as determined from the dose−response curve. [b] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50% as determined from the dose−response curve. [c] Dose−response curves were determined at five concentrations. The IC_{50} (μM) values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

In our early work on the EGFR kinase inhibitors, we found that a small lipophilic substituent (such as Cl) in the 3-position of the aniline ring was important for EGFR inhibitory activity. A similar observation was made in this work; namely, the 3-chloroaniline compounds (**8b**, **8i**) are more potent than the corresponding *des*-chloroaniline derivatives (**25b**, **8j**) in kinase assays, but this is not evident in the cell proliferation assays. However, the benefit of the 3-chloro group was abolished when an additional chloro substituent was introduced at the 5-position, namely, a 3,5-dichloro-4-benzyloxyaniline derivative (**25d** vs **8b**; **25h** vs **7f**). Replacement of the 3-chloro group of the aniline in **8b** with a 3-nitrile resulted in a much less potent compound **25e** in the kinase assays, but this effect is not as evident in the cell proliferation assays.

With respect to the substituent on the benzyloxy ring of **8b**, the 3-chloro (**8l**) and 2-chloro (**21d**) derivatives are more potent than **8b** in the kinase assays; however, their cellular activities remained similar to that of **8b**. This effect on the kinase and cellular activities parallels that seen with **7f**, where a fluoro atom is at the 3-position. What was not expected is that, unlike the potent 2-chloro analogue **21d**, the 2-fluoro counterpart **25f** was much less active in the kinase assays but remained potent in the cell proliferation assays. While the 3-fluoro derivative **7f** showed good activity, the corresponding 3,5-difluoro compound **25i** was a less effective inhibitor of both the HER-2 and EGFR enzymes. Compounds bearing 3-cyano (**25k**) or 3-methyl (**25m**) are inferior to the corresponding 3-fluoro (**7f**) and 3-chloro (**8l**) compounds. Introduction of a 4-benzyloxy

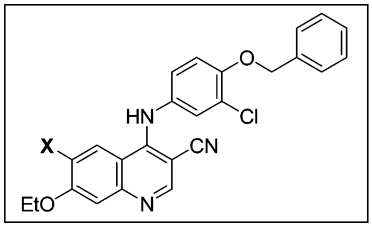
Table 4. Optimization of the C-6 Crotonamide Side Chain

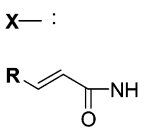


compd	R	synthetic method	kinase assays IC_{50} (μM) HER-2[a]	kinase assays IC_{50} (μM) EGFR[b]	cell proliferation assays IC_{50} (μM) A431[c]	cell proliferation assays IC_{50} (μM) SKBR3[c]	cell proliferation assays IC_{50} (μM) SW620[c]
7g	N(Me)$(CH_2)_2$OMe	1A	0.183	0.068	0.043	0.0057	0.817
7h	N(Me)$(CH_2)_2$OH	1A	0.027	0.020	0.072	0.0048	0.648
7i	N(Me)-D-glucamine	1A	0.966	0.719	3.115	0.1161	21.099
7j	N(*i*-Pr)$_2$	1A	0.028	0.030	0.258	0.0043	0.461
7k	N$[CH_2CH(OH)CH_3]_2$	1A	0.104	0.098	0.156	0.0027	1.638
7l	2-methoxymethyl-1-pyrrolidinyl	1A	0.171	0.151	0.167	0.0034	0.807
7m	*N*-piperidinyl	1A	0.025	0.031	0.244	0.0042	0.397
7n	4-OH-1-piperidinyl	1A	0.169	0.221	0.092	0.0017	0.792
7o	4,4-dihydroxy-1-piperidinyl	1A	0.058	0.282	0.063	0.0032	4.107
7p	2,6-dimethyl-1-piperidinyl	1A	0.412	0.247	0.737	0.0200	0.657
7q	*N*-morpholinyl	1A	0.059	0.020	0.037	0.0099	2.842
7r	*N*-thiomorpholinyl	1A	0.029	0.016	0.127	0.0456	5.862
7s	*N*-(4-methylpiperazinyl)	1A	0.540	0.360	0.085	0.0327	1.162
7t	*N*-azetidinyl	1A	0.040	0.026	0.158	0.0157	0.951
7u	2-carbomethoxy-1-aziridinyl	1A	0.196	0.343	0.229	0.0196	2.451
7v	1-aza-12-crown-4	1A	0.603	0.321	0.132	0.0032	0.306
8b	NMe_2	1B	0.076	0.053	0.108	0.0027	0.486
8t	*N*-pyrrolidinyl	1B	0.059	0.078	0.125	0.0053	0.309
8u	4-imidazolyl	1B	0.069	0.024	0.829	0.1485	1.727

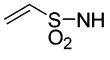
[a] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50% as determined from the dose–response curve. [b] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50% as determined from the dose–response curve. [c] Dose–response curves were determined at five concentrations. The IC_{50} (μM) values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

Table 5. Inhibitors Bearing C-6 Michael Acceptor Groups Other Than Crotonamide

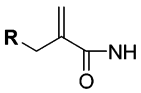




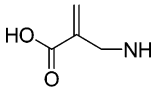
VII



VIII



XI



X

compd	series	R	kinase assays IC_{50} (μM) HER-2[a]	kinase assays IC_{50} (μM) EGFR[b]	cell proliferation assays IC_{50} (μM) A431[c]	cell proliferation assays IC_{50} (μM) SKBR3[c]	cell proliferation assays IC_{50} (μM) SW620[c]
75	VII	8-quinolinyl	1.134	0.990	13.576	4.1526	28.749
76	VII	(2-NMe_2)Ph	1.391	1.569	14.398	7.6035	32.841
77	VII	4-imidazolyl	0.227	0.255	>8.85	0.6371	>8.85
82	VIII		0.061	0.045	0.617	0.0673	1.495
83	XI	NMe_2	0.246	0.223	0.360	0.1295	0.360
84	XI	morpholinyl	0.089	0.075	0.150	0.0069	0.401
85	X		3.781	1.191	6.522	2.2685	15.501

[a] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50% as determined from the dose–response curve. [b] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50% as determined from the dose–response curve. [c] Dose–response curves were determined at five concentrations. The IC_{50} (μM) values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

substituent on the benzyloxy ring of **8b** (as shown in **8n**) is detrimental to activity.

We then replaced the phenyl ring of the benzyloxy group of **8b** with cylcohexyl (**25n**), bicyclic, or heterocyclic groups. The cyclohexyl derivative **25n** was as potent an inhibitor as **8b** for HER-2 kinase but showed 10-fold less activity against EGFR kinase. The 1-naphthyl compound (**25t**) and 2-naphthyl compound (**25u**) were slightly more potent in the kinase assays but less potent in cell proliferation assays. In the heterocyclic series, the 2-pyridinyl (**25o**), 2-furyl (**25q**), and 2-thienyl (**8o**) analogues each showed similar activities compared to **8b**.

In our earlier work,[9] we found that adding a basic dialkyamino group to the terminus of the crotonamide side chain at the 6-position of 4-anilinoquinoline-3-carbonitrile significantly increased the activity in both kinase and cell proliferation assays. On the basis of our binding model of **25o** complexed with HER-2, the water-solubilizing dimethylamino group points out of the ATP

Table 6. Correlations between log(IC_{50}) in EGFR and HER-2 Kinase Assays and Cell Proliferation Assays on Cell Lines A431, SKBR3, and SW620[a]

	log IC_{50}				
	EGFR	HER-2	A431	SKBR3	SW620
EGFR (log IC_{50})	1.000				
HER-2 (log IC_{50})	0.815	1.000			
A431 (log IC_{50})	0.365	0.281	1.000		
SKBR3 (log IC_{50})	0.335	0.255	0.934	1.000	
SW620 (log IC_{50})	0.489	0.370	0.478	0.411	1.000

[a] The Pearson product moment correlation coefficients are tabulated and are calculated using the formula

$$R = \frac{n(\sum XY) - (\sum X)(\sum Y)}{\sqrt{[n\sum X^2 - (\sum X)^2][n\sum Y^2 - (\sum Y)^2]}}$$

binding pocket toward the aqueous environment. Also, the β-carbon of the crotonamide group is 3.43 Å away from the sulfur atom of Cys 805, and the dimethylamino moiety is situated 4.46 Å from the hydrogen of the sulfhydryl group. We have proposed that the dialkylamino group of compounds such as **25o** could serve as an intramolecular base catalyst for Michael additions by extracting the proton from the nucleophilic sulfhydryl group (Cys 805 for HER-2 and Cys 773 for EGFR) through a cyclic five-membered ring mechanism.

Table 4 shows compounds where the terminal dialkylamino group of the crotonamide Michael acceptor was varied. With a few exceptions, it is evident that for most of these analogues, the kinase and cellular activities are minimally affected by the choice of the dialkylamino group. One exception is compound **7p**, which carries a 2,6-dimethyl-1-piperidinyl group. It is much less active than the corresponding compound **7m** carrying an *N*-piperidinyl group, suggesting that the steric bulk around the basic nitrogen may hinder the intramolecular base catalysis of the Michael addition of Cys 805 to form a covalent adduct. Likewise, the loss of kinase potency of **7v** may be due to the steric bulk of 1-aza-12-crown-4. However, **7v** was as potent as **8b** in the cell proliferation assays. A highly water-soluble analogue **7i** carrying an *N*-methyl-D-glucamine group was also less active in the kinase and cell proliferation assays. Compound **77**, where the Michael acceptor is terminated with an imidazole ring, showed reduced activity in both kinase and cell proliferation assays (Table 5) compared to compounds with the terminal dimethylamino group. This loss of activity is likely due to the reduced basicity of the imidazole ring of **77** compared to the imidazole in **8u**. Also, the proposed intramolecular base catalysis of the Michael addition would not be expected to occur with **77** but could occur with **8u**. Similarly, quinolino and dimethylaminophenyl substituted derivatives **75** and **76,** respectively, show reduced activities most likely due to reduced basicity. There is no major difference in activities between compounds in the crotonamide series such as **8b** and **7q** compared to the coreresponding compounds **83** and **84** in the methacrylamide series. As expected, compound **85**, lacking a basic amino substituent but carrying a carboxylic group, is a poor inhibitor of both HER-2 and EGFR. Finally, compound **82** with an ethylenesulfonamide group is as potent as **8b**. From these in vitro studies, it is clear that among the best

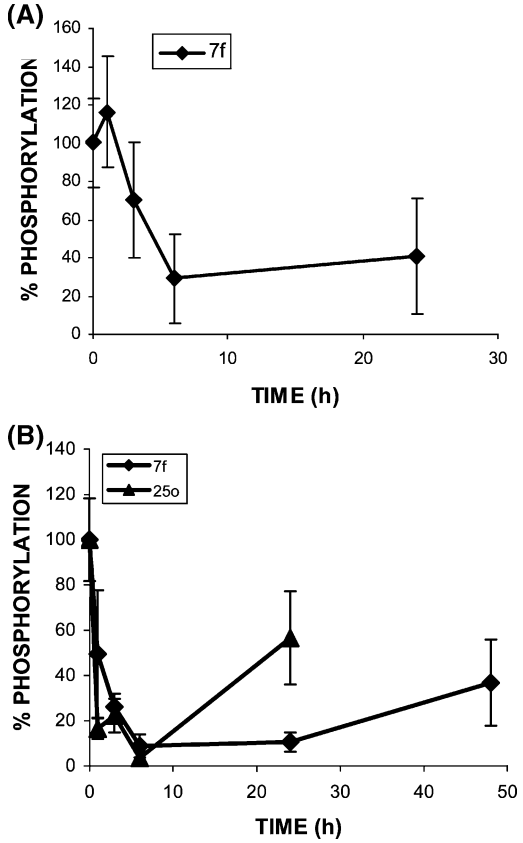


Figure 4. Inhibition of HER-2 phosphorylation in the 3T3/neu tumor model and BT474 xenograft. 3T3/neu (A) and BT474 (B) tumor-bearing mice were treated with vehicle or a single dose of compound (three to five animals per group). Tumors from control and treated mice were dissected at various times after compound administration. Protein extracts from each time point were pooled and analyzed by SDS−PAGE and immunoblotting. Extracts from individual tumors were also analyzed to determine the variability between animals. Phosphorylation of HER-2 was detected using phosphotyrosine antibodies and normalized to HER-2 levels, detected with anti HER-2 antibodies. Blots were quantified by scanning densitometry. Data for **25o** in part B are from ref 29.

dual inhibitors of HER-2 and EGFR kinases are **7f**, **7m**, **8b**, **8o**, **8t**, and **25o**. These compounds were selected for further in vivo studies.

Selectivity and Irreversible Binding to HER-2. Compound **25o** was evaluated in two additional tyrosine kinases assays, namely, KDR and Src. The IC_{50} values (0.8 μM for KDR and 1.4 μM for Src) were 14- and 24-fold greater for these kinases compared to HER-2. Compound **25o** was also evaluated against a number of serine−threonine kinases among which were Akt, cyclin D1/CDK4, cyclin E/CDK2, cyclin B1/CDK1, IKK-2, MK-2, PDK1, c-Raf, and Tpl-2, and no significant inhibitory activity was observed.[29] It is clear that **25o** is a selective inhibitor of members of the ErbB family of kinases.

In our earlier work,[9] EGFR kinase inhibitor **86** was shown to form a covalent adduct with EGFR kinase. We proposed that this is the result of a covalent interaction between the Michael acceptor group of **86** with Cys 773 located within the ATP binding pocket of EGFR. Since **25o** and **86** have identical Michael acceptor functionality and since the target cysteine residue is conserved in HER-2 (Cys 805), it is expected that **25o** will

Table 7. Comparison of in Vivo Effects (*% T/C*) of HER-2 Inhibitor Leads and EKB-569 (**86**) in Tumor Models Sensitive to HER-2 or EGFR[a]

	% T/C (*p* value)						
	3T3/*neu*		BT474		SKOV3	SUM190	A431
compd	80 mg/kg	40 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	40 mg/kg	40 mg/kg
7f	1 (<0.01)	3 (<0.01)	4 (<0.01)	41 (0.06)	12 (<0.01)	6 (<0.01)	34 (0.01)
7m	27 (<0.01)	44 (<0.01)					
8b	3 (<0.01)	13 (<0.01)	13 (<0.01)	27 (<0.01)	26 (<0.01)	29 (0.06)	47 (0.07)
8o		46 (<0.01)					
8t		36 (<0.01)					
25o	2 (<0.01)	5 (<0.01)	12 (<0.01)	34 (<0.01)	14 (<0.01)	20 (0.01)	56 (0.09)
86	5 (<0.01)	44 (<0.01)	18 (<0.01)	70 (0.16)	24 (<0.01)	32 (0.11)	6 (<0.01)

[a] Tumor cells (3T3/neu, SKOV3, SUM-190, A431) or tumor fragments (BT474) were implanted subcutaneously in female athymic (nude) mice. For hormone-dependent tumors (BT474, SKOV3), estrogen pellets were implanted 1 week prior to tumor implantation. When tumors reached a size of 90−200 mg, they were randomly assigned to treatment groups (5−10 animals per group) (staging, day 0). Compounds were formulated in 0.5% methylcellulose and 2.0% polysorbate-80 (Tween-80) and adminstered orally once daily for 20 days (10 days for 3T3/neu and A431 xenograft). For 3T3/neu tumors, dosing was started the day after tumor cell implantation (day 0) because of rapid growth. Growth of tumors was determined once a week. Tumor mass was estimated using the formula (length)(width^2)/2 and was expressed as relative tumor growth (RTG), the ratio of the tumor mass to the mass on the staging day (except 3T3/neu). The effect of the compounds on tumor growth is shown as *% T/C*, the RTG or mean tumor weight of treated group/RTG or mean tumor weight of placebo group, expressed as a percentage. Data were analyzed using a one-tailed Student's *t*-test (equal variance) after log transformation of the data. A *p* value of <0.05 indicates statistical significance of tumor inhibition. The data shown are from day 21 (3T3/neu, BT474, and A431 xenografts), day 28 (SUM-190 xenograft), and day 35 (SKOV3 xenograft), with the *p* value in parentheses. No compound-related adverse effects (death, weight loss) were observed in these studies.

irreversibly bind to HER-2. Washout experiments were designed to determine if **25o** functions as an irreversible binding inhibitor in intact BT474 cells by measuring the ability of the compound to inhibit the autophosphorylation of the receptor after removal of **25o** from the media. We found that after preincubation of cells with **25o** (5 h) followed by extensive washing to remove free drug from the media, autophosphorylation of HER-2 was still inhibited.[29]

A more direct indication of a covalent interaction with the enzyme was obtained using C-14 labeled **25o.**[29] Labeled compound was incubated with the purified cytoplasmic domain of HER-2. After denaturation, the sample was analyzed by SDS−PAGE and fluorography. A single ~95 kDa labeled band, corresponding to the HER-2 cytoplasmic domain, was observed. This radiolabeled signal was greatly diminished if the protein was preincubated with unlabeled **25o** prior to addition of radiolabeled **25o**. Similar experiments were performed by incubating C-14 labeled **25o** with intact BT474 cells. A prominent band corresponding to ~185 kDa incorporated labeled drug. Additionally, incorporation of labeled drug could be inhibited when the cells were pretreated with a 5-fold excess of cold compound prior to exposure to the labeled drug. Finally, the labeled protein could be immunoprecipitated with an anti-HER-2 antibody suggesting that the ~185 kDa species is in fact HER-2. These data strongly support our claim that **25o** functions as an irreversible binding inhibitor of HER-2 in solution and in intact cells.

HER-2 Phosphorylation in in Vivo Tumor Models. To determine whether inhibition of tumor growth in vivo was associated with a block in the target receptor function, the phosphorylation of HER-2 was evaluated in xenografts after a single oral dose of 40 mg/kg of **7f** or **25o** (Figure 4). Compound **7f** reduced HER-2 phosphorylation in 3T3/neu tumor model by 30% within 3 h as shown in Figure 4A. Inhibition reached a maximum of 71% at 6 h and was partially reversed by 24 h (59%). In BT474 xenografts, **7f** reduced HER-2 phosphorylation by 52% in 3 h as shown in Figure 4B. Maximum inhibition was observed at 6 h (87%), which was sustained at 24 h (79%) and partially reversed by 48 h (58%). Similar effects were observed with **25o**,[29] although substantial reduction of phosphorylation (84% inhibition) was observed earlier, within 1 h of compound dosing, and was partly reversed (57% inhibition) by 24 h. The sustained inhibition of phosporylation of HER-2 seen in the in vivo studies of both the 3T3/neu tumor model and BT474 xenograft is consistent with irreversible inhibition of the target enzyme HER-2.

In Vivo Efficacy. The best compounds **7f**, **7m**, **8b**, **8o**, **8t**, and **25o** based on the in vitro assays were evaluated in a number of tumor models in nude mice (Table 7). Our EGFR clinical lead **86** was also included in the testing for comparison. One model used for this evaluation involved murine fibroblast 3T3 cells transfected with HER-2/neu. Treatment was initiated the day after cell implantation in this model because of the rapid growth of the transplant. The compounds were dosed orally once a day for 10 days. After day 10, dosing was discontinued. It is evident that compounds **7f**, **8b**, and **25o** are more effective in reducing tumor growth than **86** when administered orally at 40 mg/kg. This correlates well with the higher potency of **7f**, **8b**, and **25o** against HER-2 enzyme compared to **86**. However, at a higher dose of 80 mg/kg, similar efficacy (95−99% inhibition) was observed for most of these analogues. Compounds were evaluated in three traditional tumor models that overexpress HER-2; among these were xenografts of BT-474, SKOV3, and SUM190 cells. In BT474 and SUM190 tumor models, **7f**, **8b**, and **25o** showed better efficacy and activity than **86**. For example, **86** inhibited the growth of BT474 tumors by 30% at a 10 mg/kg dose, whereas **7f**, **8b**, and **25o** showed higher inhibition (59%, 73%, and 66%, respectively) at the same dose. Since **7f**, **8b**, and **25o** inhibit EGFR kinase and proliferation of EGFR-overexpressing cells in vitro, the effect of these compounds in A431 xenografts was evaluated. Although all these compounds suppressed the growth of A431 tumor (44−66% inhibition), they were less potent than **86** (94% inhibition) in this tumor model.

Conclusions

We have described three different synthetic routes and resultant SAR of a series of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. These compounds function as irreversible binding inhibitors of HER-2 and EGFR kinases consistent with our binding models for these compounds. Significantly, some of these compounds showed better activity in HER-2 kinase and HER-2 overexpressing cell proliferation assays compared to our EGFR kinase inhibitor **86**. They also demonstrated improved potency and efficacy in HER-2 dependent in vivo models compared to **86**. On the basis of the results described herein and on extensive pharmokinetic and toxicological studies, one member of this series, **25o**, has entered clinical trials for further development as an antitumor agent in breast and other HER-2 dependent cancers.

Experimental Section

Biology. The preparation of the enzymes, the details of our kinase autophosphorylation assays, the cell proliferation assays, and the in vivo studies have been described in detail in our earlier publications.[24,29]

HER-2 Phosphorylation in Xenografts. Athymic female nude mice (Charles River Laboratories, Wilmington, MA; five animals/group) were implanted subcutaneously with 2×10^6 3T3/neu cells or a single BT474 tumor fragment (~30 mm^3). When tumors reached 200−300 mg in size, animals were given a single oral dose (40 mg/kg) of **25o** or **7f**. Tumors from control and treated animals were excised at various times up to 48 h and minced. Tumor fragments were suspended in 10 mM Tris, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, 10 μg/mL pepstatin, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 2 mM sodium vanadate, and 100 mM sodium fluoride (all chemical reagents were obtained from Sigma-Aldrich, St Louis, MO) and lysed by homogenization on ice with a polytron. After clarification by centrifugation, protein concentration in lysates was estimated using the BioRad $_{DC}$ protein assay (BioRad, Hercules, CA). An amount of 60 μg of lysate pooled from each group was analyzed by SDS−PAGE and immunoblotting with phosphotyrosine-specific antibodies (BD Biosciences PharMingen, San Diego, CA). HER-2 specific antibodies were used as controls for protein loading. Blots were quantified by scanning densitometry (FluorS MultiImage analyzer, BioRad). Extracts from individual tumors were analyzed to determine variability between animals.

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.

Preparation of Substituted Anilines. 2-(2-Chloro-4-nitrophenylsulfanyl)-5-phenyl-1,3-thiazole (39). A solution of 2.2 g (11.4 mmol) of 2-mercapto-5-phenyl-1,3-thiazole[30] in 35 mL of DMF was added dropwise to a stirring mixture of 546 mg (13.6 mmol) of 60% sodium hydride in oil suspended in 7 mL of DMF, under nitrogen. After 30 min, a solution of 2 g (11.4 mmol) of 3-chloro-4-fluorobenzene in 14 mL of DMF was added dropwise. This was stirred for 16 h at room temperature. The DMF was evaporated, and the brown residue was stirred with 100 mL of hexane and filtered. The residue was washed with three portions of water and ether and crystallized from ethyl acetate to give 3.3 g (84%) of light-yellow crystals (**39**): mp = 126−128 °C; HRMS (ESI) *m/z* 348.986 43 $(M + H)^{+1}$, Δ = −0.25 mmu; ^{1}H NMR (DMSO-d_6) δ 8.52 (s, 1H), 8.43 (d, 1H, *J* = 2.4 Hz), 8.17 (dd, 1H, *J* = 8.7, 2.4 Hz), 7.72 (d, 2H, *J* = 5.2 Hz), 7.52−7.40 (m, 4H). Anal. ($C_{15}H_9ClN_2O_2S$) C, H, N, Cl, S.

3-Chloro-4-(5-phenylthiazol-2-ylsulfanyl)phenylamine (40). A mixture of 3.13 g (8.97 mmol) of 2-(2-chloro-4-nitrophenylsulfanyl)-5-phenyl-1,3-thiazol **39** and 3.5 g (62.6 mmol) of iron powder in 37 mL of MeOH and 3.1 mL of HOAc was mechanically stirred and refluxed for 2.5 h. The hot reaction mixture was filtered, and the solvents were evaporated in vacuo. The residue was partitioned between saturated $NaHCO_3$ and EtOAc. The organic layer was separated and dried to a solid. After it was washed with ether, 2.3 g (80%) of the product (**40**) as a light-gray solid was obtained: mp = 121−123 °C; HRMS (ESI) *m/z* 319.012 51 $(M + H)^{+1}$, Δ = −0.02 mmu; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H), 7.53−7.48 (m, 3H), 7.40−7.27 (m, 3H), 6.85 (d, 1H, *J* = 1.9 Hz), 6.62 (dd, 1H, *J* = 8.5, 1.9 Hz), 6.11 (bs, 2H). Anal. ($C_{15}H_{11}ClN_2S_2$) C, H, N, S, Cl.

2-(2-Chloro-4-nitrophenoxymethyl)-1-methyl-1*H*-imidazole. The title compound was prepared from 4.60 g (0.0410 mol) of 1-methyl-1*H*-imidazol-2-yl)methanol,[31] 7.92 g (0.0454 mol) of 3-chloro-4-fluoronitrobenzene, and 1.64 g (0.0410 mol) of 60% sodium hydride in oil using the method described above for **39** to yield 7.98 g (72%) of the title compound: mp = 182−184 °C; MS (ES+) *m/z* 268.0 $(M + H)^{+1}$.

2-(2-Chloro-4-nitrophenylsulfanyl)thiazole. The title compound was prepared from 10.01 g (85.45 mmol) of thiazole-2-thiol and 15 g (85.45 mmol) of 2-chloro-1-fluoro-4-nitrobenzene using the method described above for **39** to yield 22 g (95%) of the title compound as a yellow solid: MS (ESI) *m/z* 273.0 $(M + H)^{+1}$.

2-(2-Chloro-4-nitrophenylsulfanyl)-4-phenylthiazole. To a mixture of 5.8 g (0.03 mol) of 2-mercapto-4-phenylthiazole and 1.78 g (0.033 mol) of NaOMe in 50 mL of DMF was added 5.79 g (0.033 mol) of 3-chloro-4-fluoronitrobenzene. The reaction mixture was stirred at room temperature for about 5 h. Then it was poured into about 100 mL of ice−water. The resulting solid was filtered and recrystallized from 95% EtOH to give 7.64 g (73%) of the title compound as a light-yellow solid: mp = 120−124 °C; MS (ESI) *m/z* 349.3 $(M + H)^+$.

1-(4-Amino-2-chlorobenzyl)-1*H*-imidazole (42). A solution of 10 g (39.9 mmol) of 2-chloro-4-nitrobenzyl bromide[32] and 5.4 g (79.8 mmol) of imidazole in 125 mL of THF was refluxed for 45 min. The solvent was removed, and the residue was dissolved in EtOAc. The solution was washed with H_2O and dried with $MgSO_4$. The solvent was removed, and the residue was extracted with ether. The ether solution was diluted with 2 volumes of hexane. The resulting precipitate was collected, giving 4.3 g of 1-(2-chloro-4-nitrobenzyl)-1*H*-imidazole (**41**) as a white solid. This material was mechanically stirred at reflux in 180 mL of MeOH and 52 mL of H_2O containing 8.1 g of NH_4Cl and 6.6 g of iron powder for 2 h. The mixture was filtered, and solvent was removed. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ solution. The solution was treated with activated charcoal and dried with $MgSO_4$. Solvent was removed, giving 3.9 g of the product **42** as an oil that was used without additional purification.

2-(Benzyloxy)-1,3-dichloro-5-nitrobenzene. A solution of 2.74 g (13.17 mmol) of 2,6-dichloro-4-nitrobenzene, 2.35 mL (3.38 g, 19.76 mmol) of benzyl bromide, and 10.73 g (32.92 mmol) of cesium carbonate in 67 mL of DMF was stirred at room temperature overnight. After the solvent was removed by rotary evaporation, the residue was partitioned between EtOAc and water. The organic layer was dried ($MgSO_4$) and evaporated to give a brown semisolid, which was recrystallized from EtOAc to give 1.088 g (28%) of the starting material as bright-yellow crystals: mp = 164−166 °C. The mother liquor was evaporated, and the residue was recrystallized from ether to give 2.06 g (52%) of the title compound as beige crystals: mp = 78−80 °C; HRMS (ESI+) *m/z* 298.0026, Δ = 1.1 mmu.

4-Benzyloxy-3,5-dichlorophenylamine (44). A mixture of 1.77 g (5.94 mmol) of 2-(benzyloxy)-1,3-dichloro-5-nitrobenzene, 2.33 g of iron powder, 2.2 mL of HOAc, and 92 mL of MeOH was refluxed for 3 h. After filtration, the filtrate was evaporated to a residue that was partitioned between EtOAc and saturated $KHCO_3$ solution. The organic layer was evaporated to give a light-brown oil, which was triturated with ether−hexane (1:1) to give 1.41 g (88%) of the titile compound as a beige solid: mp = 75−77 °C; HRMS (ESI+) *m/z* 268.628 45, △ = −0.62 mmu.

2-(4-Amino-2-chlorophenyl)ethanethioamide (49). A solution of 15 g (76.3 mmol) of (2-chloro-4-nitrophenyl)-acetonitrile (**48**)[19] in 100 mL of DMF was stirred at −78 °C as H_2S was introduced to saturation. To the solution was added 7.7 g (76.3 mmol) of Et_3N. The cooled mixture was placed in a steel bomb that was maintained at 80 °C for 16 h. The bomb was cooled and vented. The solution was treated with activated charcoal and filtered. The residue was diluted with ice/water, and the solid was collected and washed with H_2O and hexane. The solid was air-dried and dissolved in 150 mL of MeOH. Elemental sulfur was removed by filtration. The solvent was removed, and the product was recrystallized from EtOAc−hexane to give 8.5 g (56%) of the title compound as tan crystals: MS (ESI+) *m/z* 201.0 $(M + H)^{+1}$.

3-Chloro-4-[(4-phenyl-1,3-thiazol-2-yl)methyl]aniline (51). A solution of 4.56 g (22.92 mmol) of phenacyl bromide (**50**) and 4.6 g (22.92 mmol) of 2-(4-amino-2-chlorophenyl)-ethanethioamide (**49**) in 47 mL of EtOH was refluxed for 3 h. The mixture was poured into saturated $NaHCO_3$ and extracted with ether. The solution was dried ($MgSO_4$). Solvent was removed and the residue was chromatographed on silica gel, eluting with CH_2Cl_2−ether mixtures to yield 8.3 g (95%) of the HBr salt of the titile compound as a light-tan solid: MS (ES+) *m/z* 301.1 $(M + H)^{+1}$.

1-Benzyl-1*H*-indazol-5-amine (55). A mixture of 5 g (30.65 mmol) of 5-nitroindazole (**53**), 5.76 g (33.68 mmol) of benzyl bromide, and 4.66 g (33.72 mmol) of K_2CO_3 in 50 mL of DMF was heated at 75 °C for 4 h. After cooling, the reaction mixture was poured into 50 mL of H_2O. The solid was collected and washed with H_2O to give a mixture of two isomers: 1-*N*-benzyl-5-nitro-1*H*-indazole (major) and 2-*N*-benzyl-5-nitro-1*H*-indazole (minor). The crude mixture was dissolved in 35 mL of acetone. After filtration, 10.8 mL of H_2O was added slowly to the filtrate with stirring. After 1 h, 2.9 g (37%) 1-*N*-benzyl-5-nitro-1*H*-indazole was collected as yellow crystals. A mixture of 1-*N*-benzyl-5-nitro-1*H*-indazole (1.4 g, 5.53 mmol) and iron powder (2.25 g) in 84 mL of MeOH and 33 mL of HOAc was refluxed for 2 h. The hot reaction mixture was filtered, and the filtrate was concentrated. The residue was partitioned between EtOAc and $NaHCO_3$ solution. The organic layer was concentrated to give 1.3 g (100%) of the title compound as orange crystals (**55**): HRMS (EI) *m/z* 223.1094, △ = 1.6 mmu.

1-Benzyl-2,3-dihydro-1*H*-indol-5-ylamine (57). A solution of 3.11 g (0.0184 mmol) of 5-nitroindoline (**56**) and 2.48 mL (3.56 g, 0.0208 mol) of benzyl bromide in 35 mL of CH_3CN was refluxed for 2 h. The solvent was removed in vacuo, and the solid was stirred with saturated aqueous $NaHCO_3$. The resulting solid was chromatographed on silica gel and eluted with 1:9 EtOAc−hexanes to yield 2.83 g (58%) of 1-benzyl-5-nitro-2,3-dihydro-1*H*-indole: mp = 87−88 °C; MS (ES+) *m/z* 255.0 $(M + H)^{+1}$. A mixture of 2.54 g (0.01 mol) of 1-benzyl-5-nitro-2,3-dihydro-1*H*-indole, 2.23 g (0.04 mol) of iron powder, and 2.14 g (0.04 mol) of NH_4Cl in 65 mL of EtOH and 10 mL of H_2O was vigorously stirred and refluxed for 3 h. The reaction mixture was filtered through Celite; then the solvents were removed from the filtrate in vacuo. The residue was extracted with CH_2Cl_2, and these extracts were passed through Magnesol. The CH_2Cl_2 was removed in vacuo to give 2.04 g (91%) of **57** as an oil.

Preparation of the Michael Acceptor Groups. (*E*)-4-Iodobut-2-enoic Acid *tert*-Butyl Ester (61). A mixture of 4.42 g (20 mmol) of (*E*)-*tert*-butyl 4-bromobut-2-enoate (**60**), 6.0 g (40 mmol) of NaI, and 40 mL of acetone was refluxed for 1 h. The cooled mixture was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed successively with aqueous $NaHSO_3$, H_2O, aqueous $NaHCO_3$, and H_2O, dried, and concentrated to give an amber liquid: ^1H NMR ($CDCl_3$) δ 6.90 (dt, 1H, J = 8.3, 14.8 Hz), 5.86 (d, 1H, J = 14.8 Hz), 3.91 (d, 2H, J = 8.3 Hz), 1.48 (s, 9H).

(*E*)-4-(Pyrrolidin-1-yl)but-2-enoic Acid *tert*-Butyl Ester (62). To a stirred solution of 2.08 g (7.75 mmol) of (*E*)-4-iodobut-2-enoic acid *tert*-butyl ester (**61**) in 15.5 mL of THF at 0 °C was added 1.29 mL (15.5 mmol) of pyrrolidine. The solution was warmed to 25 °C, stirred 15 min, and partitioned between toluene and aqueous $KHCO_3$. The organic layer was washed with water, dried, and concentrated to give 1.50 g (92%) of amber oil: MS (ES+) *m/z* 212.0 $(M + H)^{+1}$.

(*E*)-4-(Pyrrolidin-1-yl)but-2-enoic Acid Hydrochloride (63). A solution of 1.49 g (7.05 mmol) of (*E*)-4-(pyrrolidin-1-yl)but-2-enoic acid *tert*-butyl ester (**62**) in 14.1 mL of 1 N HCl was refluxed for 15 min, diluted with toluene, and concentrated to give an amber solid: MS (ES+) *m/z* 155.8 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 12.7 (bs, 1H), 11.4 (bs, 1H), 6.83 (dt, 1H, J = 6.8, 14.4 Hz), 6.18 (d, 1H, J = 14.4 Hz), 3.95 (d, 2H, J = 6.8 Hz), 3.05 (m, 2H), 1.92 (m, 6H).

4-(1*H*-Imidazol-4-yl)-but-2-enoic Acid Hydrochloride (67). 1*H*-Imidazole-4-acetic acid, 1-(triphenylmethyl) methyl ester (**65**)[21] was converted to the title compound using the method reported by Takacs.[22] A solution of 6.45 g (28.8 mmol) of triethyl phosphonoacetate in 100 mL of THF was cooled to −78 °C with stirring under an inert atmosphere. A solution of 12 mL of *n*-BuLi in hexanes (2.5 M, 30 mmol) was added dropwise. After 10 min, a solution of 1*H*-imidazole-4-acetic acid, 1-(triphenylmethyl) methyl ester in 60 mL of THF was added. After the mixture was stirred for 20 min, a solution of 27.5 mL of DIBAL in CH_2Cl_2 (1 M, 27.5 mmol) was added. The mixture was stirred for 1.5 h and then allowed to slowly warm to room temperature over 2 h. Solid $Na_2SO_4(H_2O)_{10}$ was added followed by 3 mL of H_2O. The mixture was filtered, and the solvent was removed. The residue was dissolved in ethyl acetate, washed with saturated Na_2CO_3, and dried ($MgSO_4$). The product, which is a mixture of cis and trans isomers, was treated with $NaOCH_3$ in CH_3OH to convert most of the material to the trans isomer, which was purified by silica gel chromatography giving 5.9 g of intermediate (**66**). A 4.77 g portion of this ester (**66**) was hydrolyzed by dissolving it in 48 mL of CH_3OH containing 9 mL of 5 M NaOH. After being stirred for 2 h, the mixture was neutralized to pH 4−5 and extracted with ethyl acetate. The solution was dried ($MgSO_4$), and the solvent was removed. The trityl group was removed by refluxing this material in a mixture of 40 mL of H_2O, 40 mL of EtOH, and 5 mL of concentrated HCl for 1.5 h. The mixture was cooled, and solid trityl alcohol was filtered off. Solvent was removed from the filtrate and the remaining H_2O was coevaporated with toluene several times, resulting in 1.3 g of crude product HCl salt (**67**), which was used in the next step without additional purification.

3-(2-Dimethylaminophenyl)acrylic Acid (70). A solution of 18 g (120.7 mmol) of 2-(dimethylamino)benzaldehyde (**69**),[23] 12.6 g (120.7 mmol) of malonic acid, and 6 drops of piperidine in 107 mL of pyridine was heated to 110 °C for 3 h. The mixture was diluted with 600 mL of H_2O, and 30 mL of 5 N NaOH was added. The solution was washed twice with ether (washing discarded). The solution was acidified to pH 5−6, saturated with NaCl, and extracted with ethyl acetate. The extract was dried ($MgSO_4$), and the solvents were removed. The solid was dissolved in ether, activated charcoal was added, and the mixture was filtered. The solution was concentrated, and 5.8 g (25%) of solid **70** was obtained by filtration. MS (ES+) *m/z* 192.1 $(M + H)^{+1}$. Anal. ($C_{11}H_{13}NO_2$) C, H, N.

Preparation of Representative Intermediates. 4-(4-Benzyloxy-3-chlorophenylamino)-7-ethoxy-6-nitroquinoline-3-carbonitrile Hydrochloride (3g). A suspension of 2.60 g (11.15 mmol) of 4-benzyloxy-3-chloroaniline, 3.1 g (11.15 mmol) of 4-chloro-7-methoxy-6-nitroquinoline-3-carbonitrile,[9] and 0.3 g of pyridine hydrochloride in 180 mL of *i*-PrOH under nitrogen was refluxed with stirring for 8 h. When the mixture was coooled, a yellow solid was obtained, which was washed

with *i*-PrOH and ether to give 4.9 g (85%) of yellow solid: mp = 251−255 °C; HRMS (ESI) *m/z* 475.113 95 $(M + H)^{+1}$, Δ = −2.81 mmu; ^{1}H NMR (DMSO-d_6) δ 10.98 (bs, 1H), 9.33 (s, 1H), 8.88 (s, 1H), 7.67 (s, 1H), 7.58 (d, 1H, *J* = 2.0 Hz), 7.51−7.35 (m, 7H), 5.27 (s, 2H), 4.36 (q, 2H, *J* = 6.8 Hz), 1.43 (t, 3H, *J* = 6.8 Hz). Anal. ($C_{25}H_{19}N_4O_4Cl$·0.5HCl) H, N. C: calcd, 60.89; found, 59.38.

6-Amino-4-(4-benzyloxy-3-chlorophenylamino)-7-ethoxyquinoline-3-carbonitrile (4g). A 4.6 g (9 mmol) sample of **3g** in 138 mL of methanol was brought to reflux with stirring. To this was added 3.52 g of iron powder suspended in 55 mL of acetic acid, and reflux was continued for 2 h. The reaction mixture was filtered hot and left to crystallize, producing 4.9 g of yellow solid as the acetate salt. This was suspended into 700 mL of ethyl acetate and 300 mL of saturated $KHCO_3$ and stirred overnight. The aqueous phase was extracted twice with 500 mL portions of ethyl acetate, and the organic phases were combined, dried, and evaporated to a volume of about 100 mL. This yielded 2.73 g (68%) of product in three crops as off-white crystals: mp = 237−239 °C; HRMS (ESI) *m/z* 445.141 15 $(M + H)^{+1}$, Δ = −1.43 mmu; ^{1}H NMR (DMSO-d_6) δ 9.10 (s, 1H), 8.30 (s, 1H), 7.52−7.48 (m, 2H), 7.46−7.32 (m, 3H), 7.28−7.19 (m, 4H), 5.44 (bs, 2H), 5.19 (s, 2H), 4.23 (q, 2H, *J* = 6.9 Hz), 1.45 (t, 3H, *J* = 6.9 Hz). Anal. ($C_{25}H_{21}N_4O_2Cl$) C, H, N.

Synthesis Using Method 1A. (*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-[(2-hydroxyethyl)(methyl)amino]-2-butenamide (7h). To a suspension of 6-amino-4-(4-benzyloxy-3-chlorophenylamino)-7-methoxyquinoline-3-carbonitrile (1 g, 2.25 mmol) (**4**) in 12 mL of THF at 0 °C, was added Hunig's base (0.78 mL, 4.5 mmol), followed by dropwise addition of 4-bromocrotonyl chloride (0.33 mL, 2.9 mmol) in 35 min. After the mixture was stirred for 2 h at 0 °C, the solvent was evaporated. The residue was partitioned between EtOAc and saturated sodium bicarbonate solution. The organic layer was dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by column chromatography using a gradient of ethyl acetate in methylene chloride to yield 0.79 g (62% yield) of a yellow solid as a 1:1 mixture of (*E*)-*N*-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-bromo-2-butenamide and (*E*)-*N*-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-chloro-2-butenamide. MS (ES+) *m/z* 593 $(M + H)^{+1}$, 547 $(M + 2H)^{+1}$.

A 0.18 g (0.316 mmol) portion of the bromo/chloro mixture prepared above was dissolved in 1.8 mL of DMF and cooled to 0 °C. Sodium iodide (24 mg, 0.158 mmol) was added, follwed by methoxyethylmethylamine (0.28 mL, 3.16 mmol). The reaction mixture was stirred overnight at room temperature. The reaction solution was evaporated, and the residue was partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and evaporated to an oil. Chromatography of the crude residue on a short column, eluting with EtOAc, then 5% MeOH/EtOAc, 10% MeOH/EtOAc, and 15% MeOH/EtOAc yielded 135 mg (71%) of yellow solid: mp = 55−70 °C; ^{1}H NMR (DMSO-d_6) δ 9.62 (bs, 1H), 9.49 (s, 1H), 8.96 (s, 1H), 8.47 (s, 1H), 7.49 (d, 2H, *J* = 6.9 Hz), 7.44−7.32 (m, 5H), 7.27−7.18 (m, 2H), 6.8−6.73 (m, 1H), 6.58 (d, 1H, *J* = 15.4 Hz), 5.21 (s, 2H), 4.31 (q, 2H, *J* = 6.9 Hz), 3.43 (t, 2H, *J* = 5.8 Hz), 3.33 (s, 2H), 3.23 (s, 3H), 3.19 (d, 2H, *J* = 5.5 Hz), 2.2 (s, 3H), 1.46 (t, 3H, *J* = 6.9 Hz). Anal. (C_{33} $H_{34}ClN_5O_4$·0.3H_2O) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(4-pyridinyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (7b). The title compound was prepared using the method described above for **7h**: MS (ES+) *m/z* 543.4 $(M + H)^{+1}$, 272.2 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 10.08 (s, 1H), 9.77 (s, 1H), 8.95 (s, 1H), 8.76 (s, 1H), 7.66 (m, 2H), 7.51 (m, 2H), 7.40 (d, 1H, *J* = 2.1 Hz), 7.22 (dd, 1H, *J* = 8.4 Hz, 2.1 Hz), 6.84 (m, 2H), 6.23 (m, 2H), 4.36 (q, 2H, *J* = 6.9 Hz), 3.85 (m, 2H), 2.52 (s, 6H), 1.44 (t, 3H, *J* = 6.9 Hz). Anal. $C_{29}H_{27}ClN_6O_3$·4H_2O) C, N. H: calcd, 5.74; found, 5.20.

(*E*)-*N*-(4-{[3-Chloro-4-(1,3-thiazol-2-ylsulfanyl)phenyl]amino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (7d). The title compound was prepared using the method described above for **7h**: MS (ES+) *m/z* 551.1 $(M + H)^{+1}$, 276.2 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δδ 9.97 (s, 1H), 9.83 (s, 1H), 9.04 (s, 1H), 8.76 (s, 1H), 7.71 (m, 3H), 7.53 (s, 1H), 7.40 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 6.76 (m, 2H), 4.07 (s, 3H), 3.49 (m, 2H), 2.47 (s, 6H). Anal. ($C_{26}H_{23}ClN_6O_2S_2$·1.4H_2O) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(3-fluorobenzyloxy)-phenylamino]-3-cyano-7-ethoxy-6-quinolinyl}-(dimethylamino)-2-butenamide (7f). The title compound was prepared using the method described above for **7h** to yield the product (29%) as a yellow glass after silica gel chromatography: HRMS (ESI) *m/z* 574.199 79 $(M + H)^{+1}$, Δ = −1.79 mmu; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.50 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.51−7.43 (m, 1H), 7.39−7.30 (m, 4H), 7.26−7.16 (m, 3H), 6.82−6.73 (m, 1H), 6.62−6.57 (m, 1H), 5.25 (s, 2H), 4.31 (q, 2H, *J* = 7.0 Hz), 3.07 (d, 2H, *J* = 5.6 Hz), 2.18 (s, 6H), 1.47 (t, 3H, *J* = 7.0 Hz). Anal. ($C_{31}H_{29}ClFN_5O_3$·1H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-[methyl(2,3,4,5,6-pentahydroxyhexyl)amino]-2-butenamide (7i). The title compound was prepared using the method described above for **7h**. The crude product was purified by preparative TLC developed with EtOAc−MeOH−NH_4OH = 40:4:2. The resulting material was dissolved in acetone and filtered through Celite to obtained 125 mg (20%) of light-yellow solid: mp = 72 °C; HRMS (ESI) *m/z* 705.256 68 $(M + H)^{+1}$, Δ = −0.28 mmu; ^{1}H NMR (DMSO-d_6) δ 9.59 (s, 1H), 9.53 (s, 1H), 8.84 (s, 1H), 8.46 (s, 1H), 7.49 (d, 2H, *J* = 6.9 Hz), 7.44−7.35 (m, 7H), 7.27−7.19 (m, 2H), 5.21 (s, 2H), 4.45 (s, 1H), 4.29 (q, 4H, *J* = 6.9 Hz), 4.0 (m, 2H), 3.84 (bs, 1H), 3.56 (m, 2H) 3.48 (bs, 1H), 3.25 (d, 2H, *J* = 7.4 Hz), 3.11 (m, 1H), 2.77 (m, 2H), 2.34 (s, 1H), 2.13 (s, 3H), 1.46 (t, 3H, *J* = 6.8 Hz). Anal. ($C_{36}H_{40}ClN_5O_8$·2.5H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(1-piperidinyl)-2-butenamide (7m). The title compound was prepared using the method described above for **7h** except NaI was replaced by 0.153 mL (0.88 mmol) of Hunig's base. Chromatography of the crude residue on a short column, eluting with 10% MeOH/EtOAc, yielded 273 mg (52%) of yellow glass: mp = 105−107°C; HRMS (ESI) *m/z* 595.235 15 $(M + H)^{+1}$, Δ = −0.54 mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.51 (s, 1H), 8.94 (s, 1H), 8.47 (s, 1H), 7.49 (d, 2H, *J* = 6.9 Hz), 7.44−7.35 (m, 5H), 7.27−7.18 (m, 2H), 6.8−6.73 (m, 1H), 6.58 (d, 1H, *J* = 15.3 Hz), 5.21 (s, 2H), 4.31 (q, 2H, *J* = 6.9 Hz), 3.11 (bs, 2H), 2.36 (bs, 4H), 1.51 (d, 4H, *J* = 5 Hz), 1.46 (t, 3H, *J* = 6.9 Hz), 1.40 (bs, 2H). Anal. ($C_{34}H_{34}ClN_5O_3$·2.3H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-[(2*R*,6*S*)-2,6-dimethylpiperidinyl]-2-butenamide (7p). The title compound was prepared using the method described above for **7h** except NaI was replaced by Hunig's base. Chromatography of the crude residue was done on a short column, eluting with EtOAc, 5% MeOH/EtOAc,EtOAc/MeOH/NH_4OH = 40:4:1. The resulting material was purified in preparative plates, developing in EtOAc/MeOH/NH_4OH = 40:4:1 to yield a light-yellow solid (43% yield): mp = 98−99 °C; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.49 (s, 1H), 8.93 (s, 1H), 8.47 (s, 1H), 7.49 (d, 2H, *J* = 6.9 Hz), 7.44−7.35 (m, 5H), 7.27−7.18 (m, 2H), 6.98−6.91 (m, 1H), 6.61 (d, 1H, *J* = 15.3 Hz), 5.21 (s, 2H), 4.31 (q, 2H, *J* = 6.9 Hz), 3.45 (d, 2H, *J* = 5.8 Hz), 2.41 (bs, 2H), 1.54 (m, 3H), 1.46 (t, 3H, *J* = 6.9 Hz), 1.28−1.14 (m, 3H), 1.05 (d, 6H, *J* = 6.1 Hz). Anal. ($C_{36}H_{38}ClN_5O_3$·1.05H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(4-morpholinyl)-2-butenamide (7q). The title compound was prepared using the method described above for **7h.** Chromatography of the crude residue on a short column, eluting with EtOAc and then 5% MeOH/EtOAc and 10% MeOH/EtOAc, yielded a yellow solid (75% yield): HRMS (ESI) *m/z* 597.2144 $(M + H)^{+1}$, Δ = −0.95 mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (bs, 1H), 9.52 (s, 1H), 8.95 (s, 1H), 8.47 (s, 1H), 7.49 (d, 2H, *J* = 6.9 Hz), 7.44−7.32 (m, 5H), 7.24−7.18 (m, 2H), 6.79−6.72 (m, 1H), 6.59 (d, 1H, *J* = 15.4 Hz), 5.21 (s, 2H), 4.32 (q, 2H, *J* = 6.9 Hz), 3.59 (t, 4H, *J* = 4.4 Hz), 3.14 (d,

2H, J = 5.5 Hz), 2.38 (s, 4H), 1.46 (t, 3H, J = 6.9 Hz). Anal. (C_{33} $H_{32}ClN_5O_4 \cdot 0.35H_2O$) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(1,4,7-trioxa-10-azacyclododecan-10-yl)-2-butenamide (7v). The title compound was prepared using the method described above for **7h** except NaI was replaced by Hunig's base. Chromatography of the crude residue on a short column, eluting with 5% MeOH/EtOAc, 10% MeOH/EtOAc, EtOAc/MeOH/NH_4OH = 40:4:1, yielded a yellow glass (82% yield): mp = 82−83 °C; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.38 (s, 1H), 8.98 (s, 1H), 8.46 (s, 1H), 7.49 (d, 2H, J = 6.9 Hz), 7.44−7.32 (m, 5H), 7.27−7.18 (m, 2H), 6.82−6.75 (m, 1H), 6.66 (d, 1H, J = 15.3 Hz), 5.21 (s, 2H), 4.31 (q, 2H, J = 6.9 Hz), 3.56 (t, 12H, J = 4.2 Hz), 3.28 (d, 2H, J = 5.3 Hz), 2.65 (t, 4H, J = 4.5 Hz), 1.46 (t, 3H, J = 6.9 Hz). Anal. ($C_{37}H_{40}ClN_5O_6 \cdot 0.5H_2O$) C, H, N.

Synthesis Using Method 1B. (*E*)-*N*-{4-[3-Chloro-4-(5-phenylthiazol-2-ylsulfanyl)phenylamino]-3-cyano-7-methoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8d). A 0.044 mL (0.505 mmol) portion of oxalyl chloride was added to 93 mg (0.562 mmol) of 4-(dimethylamino)-1-ylbut-2-enoic acid hydrochloride in 1.2 mL of CH_3CN. This was followed by the addition of a trace amount of DMF. This solution was heated in an oil bath at 60 °C for 30 min after gas evolution began. Then the orange reaction solution was concentrated to about $^1/_4$ of its volume in vacuo without the application of heat. This solution was then cooled in an ice bath, and 145 mg (0.281 mmol) of 6-amino-4-[3-chloro-4-(5-phenylthiazol-2-ylsulfanyl)-phenylamino]-7-methoxyquinoline-3-carbonitrile in 1.2 mL of *N*-methylpyrrolidinone was added dropwise. Cooling and stirring were continued for 2 h, then the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution. The resulting brown precipitate was collected and chromatographed on silica gel. The column was eluted with a gradient of EtOAc to 1:9: 0.2 MeOH−CH_2Cl_2−Et_3N. The product was washed with ether to give 88 mg (50%) of **8d**: HRMS (ESI) m/z 627.138 10 (M + H)$^{+1}$, Δ = −2.74 mmu; ^{1}H NMR (DMSO-d_6) δ 9.99 (bs, 1H), 9.72 (s, 1H), 9.09 (s, 1H), 8.75 (s, 1H), 8.15 (s, 1H), 7.78 (d, 1H, J = 8.5 Hz), 7.57−7.52 (m, 3H), 7.44−7.30 (m, 4H), 7.20 (dd, 1H, J = 8.5 Hz, J = 2.3 Hz), 6.83−6.62 (m, 2H), 4.07 (s, 3H), 3.07 (d, 2H, J = 5.5 Hz), 2.19 (s, 6H). Anal. ($C_{32}H_{27}ClN_6O_2S_2 \cdot 3.2H_2O \cdot 0.5$EtOAc) C, H, N.

(*E*)-*N*-[4-(4-Benzyloxy-3-chlorophenylamino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (8b). The title compound was prepared using the method described above for **8d**: pale-yellow crystals (62% yield); mp = 108−112 °C; HRMS (ESI) m/z 1111.414 43 (2M + H)$^{+1}$, Δ = −0.29 mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.49 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.53−7.48 (m, 2H), 7.45−7.35 (m, 5H), 7.27−7.18 (m, 2H), 6.81−6.57 (m, 2H), 5.22 (s, 2H), 4.31 (q, 2H, J = 5.2 Hz), 3.07 (d, 2H, J = 3.7 Hz), 2.18 (s, 6H), 1.47 (t, 3H, J = 5.2 Hz). Anal. ($C_{31}H_{30}ClN_5O_3 \cdot 1.25H_2O$) C, H, N.

(*E*)-*N*-(4-{3-Chloro-4-[(4-phenyl-1,3-thiazol-2-yl)sulfanyl]-anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (8e). The title compound was prepared using the method described above for **8d**: 55% yield: MS (ES+) m/z 641.3 (M + H)$^{+1}$, 321.2 (M + 2H)$^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.98 (s, 1H), 9.51 (s, 1H), 9.06 (s, 1H), 8.75 (s, 1H), 8.00 (s, 1H), 7.92 (s, 1H), 7.89 (s, 1H), 7.79 (d, 1H), 7.46 (m, 3H), 7.36 (d, 2H, J = 7.23 Hz), 7.18 (dd, 1H, J = 8.52 Hz, J = 2.31 Hz), 6.80 (dt, 1H, J = 15.33 Hz, J = 5.79 Hz), 6.64 (d, 1H, J = 15.45 Hz), 4.36 (q, 2H, J = 6.93 Hz), 3.09 (d, 2H, J = 5.55 Hz), 2.19 (s, 6H), 1.49 (t, 3H, J = 6.93 Hz). Anal. ($C_{33}H_{29}ClN_6O_2S_2 \cdot H_2O$) C, H, N.

(*E*)-*N*-(4-{3-Chloro-4-[(4-phenyl-1,3-thiazol-2-yl)-methyl]anilino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (8f). The title compound was prepared using the method described above for **8d**: MS (ES+) m/z 609.2 (M + H)$^{+1}$, 305.2 (M + 2H)$^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 9.69 (s, 1H), 9.03 (s, 1H), 8.63 (s, 1H), 7.94 (m, 3H), 7.49 (m, 4H), 7.35 (m, 2H), 7.19 (dd, 1H, J = 2.2 Hz, J = 8.3 Hz), 6.79 (m, 1H), 6.63 (d, 1H, J = 15.4 Hz), 4.49 (s, 2H), 4.01 (s, 3H), 2.20 (s, 6H), 3.10 (d, 2H, J = 5.6 Hz). Anal. ($C_{33}H_{29}ClN_6O_2S \cdot 1.25$ H_2O) C, H, N.

(*E*)-*N*-{4-[(1-Benzyl-1*H*-indol-5-yl)amino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8g). The titile compound was prepared using the method described above for **8d**. The crude product was chromatographed on silica gel. The column was eluted with 1:49 MeOH−CH_2Cl_2 to give 175 mg (23%) of **8g**: mp = 209−212 °C; MS (ES+) m/z 545.0 (M + H)$^{+1}$, 272.9, 273.2 (M + 2H)$^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 9.50 (s, 1H), 8.97 (s, 1H), 8.35 (s, 1H) 7,49 (m, 3H), 7.28 (m, 4H), 7.18 (m, 2H), 7.04 (dd, 1H, J = 1.83 Hz, J = 8.67 Hz), 6.77 (dt, 1H, J = 5.91 Hz, J = 15.36 Hz), 6.54 (m, 2H), 5.45 (s, 2H), 4.29 (q, 2H, J = 6.9), 3.08 (d, 2H, J = 5.5 Hz), 2.18 (s, 6H), 1.46 (t, 3H, J = 6.9 Hz). Anal. ($C_{33}H_{32}N_6O_2 \cdot 0.5H_2O$) C, H, N.

(*E*)-*N*-{4-[(1-Benzyl-1*H*-indazol-5-yl)amino]-3-cyano-7-methoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8h). The title compound was prepared using the method described above for **8d**: MS (ES+) m/z 532.1 (M + H)$^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 9.70 (s, 1H), 9.5 (s, 1H), 8.45 (s, 1H), 8.1 (s, 1H), 7.7 (m, 2H), 7.4 (s, 1H), 7.3 (m, 4H), 7.2 (m, 2H), 6.6 (m, 2H), 5.7 (s, 2H), 4.02 (s, 3H), 3.08 (d, 2H, J = 5.0 Hz), 2.18 (s, 6H), 2.2 (s, 6H). Anal. ($C_{31}H_{29}N_7O_2 \cdot 1.5H_2O$) C, H, N.

(*E*)-*N*-[4-(4-Benzyloxy)anilino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (8i). The title compound was prepared using the method described above for **8d**. The crude product was recrystallized from EtOAc−1% MeOH to produce 587 mg (54%) of beige crystals: mp = 131−133 °C; HRMS (EI) m/z 541.1891, Δ = −1.0 mmu; ^{1}H NMR (DMSO-d_6) δ 9.64 (bs, 2H), 8.97 (s, 1H), 8.45 (s, 1H), 7.51−7.44 (m, 2H), 7.42−7.32 (m, 5H), 7.26−7.16 (m, 2H), 6.8−6.56 (m, 2H), 5.21 (s, 2H), 4.02 (s, 3H), 3.06 (d, 2H, J = 5.4 Hz), 2.17 (s, 6H). Anal. ($C_{30}H_{28}ClN_5O_3 \cdot 3H_2O$) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)anilino]-3-cyano-7-methoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8j). The title compound was prepared using the method described above for **8d**. Chromatography of the crude residue on a column, eluting with 10% MeOH−EtOAc, 15% MeOH−EtOAc, and EtOAc−MeOH−Et_3N = 40:4:1 yielded a yellow solid (38% yield): mp = 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 9.64 (bs, 1H), 9.56 (s, 1H), 8.95 (s, 1H), 8.41 (s, 1H), 7.49 (d, 2H, J = 6.8 Hz), 7.43−7.33 (m, 4H), 7.21 (d, 2H, J = 6.9 Hz), 7.04 (dd, 2H, J = 2.1 Hz, J = 6.8 Hz), 6.8−6.71 (m, 1H), 6.57 (d, 1H, J = 15.4 Hz), 5.11 (s, 2H), 4.01 (s, 3H), 3.04 (d, 2H, J = 7.4 Hz), 2.17 (s, 6 H); HRMS (ESI) m/z 508.234 32 (M + H)$^{+1}$, Δ = −0.33 mmu. Anal. ($C_{30}H_{29}N_5O_3 \cdot 1.5H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(3-chlorobenzyloxy)phenylamino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8l). The title compound was prepared using the method described above for **8d.** The crude product was chromatographed to yield a yellow glassy solid: HRMS (ESI) m/z 590.169 20 (M + H)$^{+1}$, Δ = −2.83 mmu; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.50 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.56 (s, 1H), 7.47−7.39 (m, 5H), 7.26−7.17 (m, 2H), 6.82−6.57 (m, 2H), 5.24 (s, 2H), 4.31 (q, 2H, J = 5.8 Hz), 3.08 (d, 2H, J = 3.6 Hz), 2.18 (s, 6H), 1.47 (t, 3H, J = 7.0 Hz). Anal. ($C_{31}H_{29}Cl_2N_5O_3 \cdot 2H_2O \cdot 0.1$EtOAc) C, H, N.

(*E*)-*N*-[4-(4-{[4-(Benzyloxy)benzyl]oxy}-3-chloroanilino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (8n). This compound was prepared as a light-yellow solid (61%) using the method described for **8d**: MS (ES+) m/z 661.8 (M + H)$^{+1}$, 331.5 (M + 2H)$^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.60 (s,1H), 9.51 (s,1H), 8.96 (s,1H), 8.47 (s,1H), 7.48−7.33 (m, 9H), 7.27−7.18 (m, 2H), 7.05 (d, 2H, J = 8.7 Hz), 6.82−6.73 (m, 1H), 6.59 (d, 1H, J = 14.7 Hz), 5.12 (s, 4H), 4.30 (q, 2H, J = 6.9 Hz), 3.07 (d, 2H, J = 6 Hz), 2.18 (s, 6H), 1.46 (t, 3H, J = 6 Hz). Anal. ($C_{38}H_{36}ClN_5O_4 \cdot H_2O$) C, H, N.

(2*E*)-*N*-{4-[3-Chloro-4-(2-thienylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8o). This compound was prepared using the method described for **8d**. Chromatography of the crude material on a silica gel pack, eluting with 5% MeOH−$CHCl_3$, yielded a tan foam (51% yield): HRMS (ESI) m/z 561.160 24 (M + H)$^{+1}$, Δ

= −0.54 mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (bs, 1H), 9.49 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.58 (dd, 1H, J = 1.1 Hz, J = 5 Hz), 7.37 (d, 2H, J = 10 Hz), 7.29 (d, 1H, J = 8.8 Hz), 7.24−7.17 (m, 2H), 7.06−7.04 (m, 1H), 6.81−6.74 (m, 1H), 6.59 (d, 1H, J = 15.4 Hz), 5.38 (s, 2H), 4.30 (q, 2H, J = 6.9 Hz), 3.07 (d, 2H, J = 5.1 Hz), 2.17 (s, 6H), 1.47 (t, 3H, J = 6.9 Hz). Anal. (C_{29} $H_{28}ClN_5O_3$ S) C, H, N.

(*E*)-*N*-(4-{3-Chloro-4-[(1*R*)-2,3-dihydro-1*H*-inden-1-yloxy]-anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (8p). This compound was prepared using the method described for **8d**: MS (ES+) *m/z* 581.9 $(M + H)^{+1}$, 291.5 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 1H), 9.50 (s, 1H), 8.99 (s, 1H), 8.49 (s, 1H), 7.43−7.35 (m, 6H), 7.25 (m, 2H), 6.79 (m, 1H), 6.60 (d, 1H, J = 14.5 Hz), 5.92 (m, 1H), 4.31 (q, 2H, J = 5.2 Hz), 3.11 (m, 1H), 3.08 (d, 2H, J = 6 Hz), 2.86 (m, 1H), 2.54 (m, 1H), 2.18 (s, 6H), 2.09 (m, 1H), 1.47 (t, 3H, J = 5.2 Hz). Anal. ($C_{33}H_{32}ClN_5O_3$·1.2H_2O) C, H, N.

(*E*)-*N*-(4-{3-Chloro-4-[(1*S*)-2,3-dihydro-1*H*-inden-1-yloxy]-anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (8q). This compound was prepared using the method described for **8d**: MS (ES+) *m/z* 581.9 $(M + H)^{+1}$, 291.5 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 1H), 9.50 (s, 1H), 8.98 (s, 1H), 8.49 (s, 1H), 7.35 (m, 6H), 7.25 (m, 2H), 6.79 (m, 1H), 6.60 (d, 1H, J = 14.5 Hz), 5.92 (m, 1H), 4.31 (q, 2H, J = 5.2 Hz), 3.11 (m, 1H), 3.08 (d, 2H, J = 6.9 Hz), 2.86 (m, 1H), 2.54 (m, 1H), 2.18 (s, 6H), 2.09 (m, 1H), 1.47 (t, 3H, J = 5.2 Hz). Anal. ($C_{33}H_{32}ClN_5O_3$·1.2H_2O) C, H, N.

(*E*)-*N*-{4-[3-(Benzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (8r). This compound was prepared using the method described for **8d**: MS (ES+) *m/z* 522.1 $(M + H)^{+1}$, 261.8 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.60 (s, 1H), 9.80 (s, 1H), 9.00 (s, 1H), 8.45 (s, 1H), 7.41(m, 6H), 7.30 (t, 1H, J = 4.7 Hz), 6.82 (m, 4H), 6.60 (d, 1H, J = 15.5 Hz), 5.10 (s, 2H), 4.34 (q, 2H, J = 6.8 Hz), 2.17 (s, 6H), 3.12 (d, 2H, J = 6.8 Hz), 1.47 (t, 3H, J = 6.8 Hz). Anal. ($C_{31}H_{31}N_5O_3$·H_2O) C, H, N.

(*E*)-*N*-(4-{4-[Benzyl(methyl)amino]-3-chloroanilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (8s). This compound was prepared using the method described for **8d**: MS (ES+) *m/z* 569.1 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 11.35−11.19 (m, 2H), 10.0 (s, 1H), 9.17 (s, 1H), 9.05 (s, 1H), 7.67 (s, 1H), 7.60 (s, 1H), 7.35−7.25 (m, 6H), 7.18 (d, 1H, J = 7.8 Hz), 6.96−6.87 (m, 1H), 6.8 (d, 1H, J = 16.5 Hz), 4.33 (q, 2H, J = 7.5 Hz), 4.26 (s, 1H), 3.95 (t, 2H, J = 3.9 Hz), 2.74 (s, 6H), 2.65 (s, 3H), 1.50 (t, 3H, J = 3.9 Hz). Anal. ($C_{32}H_{33}ClN_6O_2$·3HCl·1.3H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(pyrrolidin-1-yl)-2-butenamide (8t). To a stirred suspension of 1.20 g (6.26 mmol) of (*E*)-4-(pyrrolidin-1-yl)but-2-enoic acid hydrochloride (**63**) in 14 mL of MeCN at 25 °C was added 0.01 mL of *N*-methyl-2-pyrrolidinone (NMP, catalyst) followed by 0.55 mL (∼6.26 mmol) of oxalyl chloride. The resulting mixture was stirred at 55 °C for 10 min, concentrated to a volume of ∼5 mL at <15 °C, and cooled at 0 °C as 7.0 mL of NMP was added. To this solution was added a solution of 1.56 g (3.5 mmol) of **4g** in 7.0 mL of NMP. The solution was warmed to 25 °C, stirred for 1 h, and treated with aqueous K_2CO_3. The resulting precipitate was filtered, washed with H_2O, and dried. The product was purified by short column chromatography on silica gel with final elution by 25:25:2:1 CH_2Cl_2−EtOAc−MeOH−Et_3N to give 1.63 g (80%) of amber glass: MS (ES+) *m/z* 582.2, 584.2 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.52 (s, 1H), 8.96 (s, 1H), 8.47 (s, 1H), 7.51 (d, 1H, J = 1.6 Hz), 7.49 (s, 1H), 7.45−7.15(m, 7H), 6.83 (dt, 1H, J = 5.6, J = 15.6 Hz), 6.62 (d, 1H, J = 15.6 Hz), 5.22 (s, 2H), 4.31 (q, 2H, J = 7.0 Hz), 3.33 (d, 2H, J = 5.6 Hz), 2.56 (m, 4H), 1.74 (m, 4H), 1.47 (t, 3H, J = 7.0 Hz). Anal. ($C_{33}H_{32}ClN_5O_3$·0.75MeOH) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(1*H*-imidazol-4-yl)-2-butenamide (8u). This compound was prepared by the method described above for **8d** using 4-(1*H*-imidazol-4-yl)but-2-enoic acid hydrochloride (**67**): MS (ES+) *m/z* 579.3 $(M + H)^{+1}$, 296.2 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 12.06 (bs, 1H), 9.61 (s, 1H), 9.45 (s, 1H), 8.87 (s, 1H), 8.46 (s, 1H), 7.60 (s, 1H), 7.50−7.32 (m, 8H), 7.23 (m, 2H), 7.04 (s, 1H), 6.48 (d, 1H, J = 15.8 Hz), 5.21 (s, 2H), 4.27 (q, 2H, J = 5.7 Hz), 3.36 (s, 2H), 1.39 (t, 3H, J = 5.7). Anal. ($C_{32}H_{27}ClN_6O_3$·1.4H_2O) C, H. N: calcd, 13.91; found, 12.78.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-3-(8-quinolinyl)-2-propenamide (75). The acid chloride hydrochloride salt of 3-quinolin-8-ylacrylic acid[33] was prepared by refluxing 1.61 g (8.09 mmol) of the acid in 20.6 mL of $SOCl_2$. After removal of the excess reagent, the resulting solid was washed several times with dry ether. This material was then used to prepare the title compound by the method described above for **8d**: MS (ES+) *m/z* 627.40 $(M + H)^{+1}$, 313.7 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.67 (s, 1H), 9.66 (s, 1H), 9.09 (s, 1H), 9.00 (m, 1H), 8.49 (s, 1H), 8.44 (dd, 1H, J = 1.7 Hz, J = 8.3 Hz), 8.20 (d, 1H, J = 7.20 Hz), 8.08 (d, 1H, J = 7.2 Hz), 7.73 (t, 1H, J = 7.7 Hz), 7.64 (m, 1H), 7.50−7.22 (m, 11H), 5.23 (s, 2H), 4.36 (q, 2H, J = 5.7 Hz), 1.51 (t, 3H, J = 5.7 Hz). Anal. ($C_{37}H_{28}ClN_5O_3$·0.66H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-3-[2-(dimethylamino)phenyl]-2-propenamide (76). The acid chloride of 3-[2-(dimethylamino)-phenyl]acrylic acid (**70**) was prepared using $(COCl)_2$ as described above and was utilized by the method described above for **8d** to prepare the title compound: MS (ES+) *m/z* 618.0 $(M + H)^{+1}$, 309.6 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 1H), 9.53 (s, 1H), 9.12 (s, 1H), 8.48 (s, 1H), 7.93 (d, 1H, J = 15.8 Hz), 7. 61 (m, 1H), 7.52−7.08 (m, 13H), 5.22 (s, 2H), 4.35 (q, 2H, J = 6.9 Hz), 2.69 (s, 6H), 1.50 (t, 3H, J = 6.9 Hz). Anal. ($C_{36}H_{32}ClN_5O_3$·5H_2O) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-3-(1*H*-4-imidazolyl)acrylamide (77). This was prepared from 0.44 g (1.0 mmol) of 6-amino-4-[4-(benzyloxy)-3-chlorophenylamino]-7-ethoxyquinoline-3-carbonitrile (**4g**) and 3-(1*H*-imidazol-4-yl)acryloyl chloride hydrochloride as described above for **8d**. The acid chloride was prepared from 0.235 g (1.7 mmol) of the corresponding acid and 8.5 mL of $SOCl_2$ at reflux temperature followed by evaporation. The crude product was triturated with hot MeOH to give 376 mg (67%) of light-yellow solid: mp = 196−201 °C (dec); MS (ES+) *m/z* 283.1, 284.1 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 12.3 (bs, 1H), 9.62 (bs, 1H), 9.50 (s, 1H), 9.02 (s, 1H), 8.47 (s, 1H), 7.76 (s, 1H), 7.45−7.30 (m, 10H), 7.23 (m, 1H), 7.08 (d, 1H, J = 15.1 Hz), 5.22 (s, 2H), 4.31 (q, 2H, J = 6.8 Hz), 1.49 (t, 3H, J = 6.8 Hz). Anal. ($C_{31}H_{25}ClN_6O_3$·2H_2O·H_2CO_3) C, H, N.

Synthesis Using Method 2. 2-Acetamido-5-nitrophenol (10). To a stirred suspension of 400 g (2.6 mol) of 2-amino-5-nitrophenol (**9**) in 1.6 L of HOAc at 60 °C was added 368 mL (3.9 mol) of Ac_2O over 1.5 h. The reaction mixture was stirred at 60 °C for 2 h. More Ac_2O was added until less than 1% of 2-amino-5-nitrophenol (**9**) was present, as determined by HPLC. An amount of 2 L of H_2O was added to the reaction mixture after it was cooled to 25 °C. The suspension was stirred in H_2O for 1 h, filtered, washed with 2 × 640 mL of H_2O, washed with 2 × 640 mL of heptane, and dried in an oven to yield 486.7 g (96%) product (**10**): mp > 205 °C.

4-Acetamido-3-ethoxynitrobenzene (11). To a stirred suspension of 400 g (2.04 mol) of 2-acetamido-5-nitrophenol (**10**) and 790 g (5.72 mol) of K_2CO_3 in 2.0 L of DMF at 60−62 °C was added 294 g (2.70 mol) of EtBr over 1 h. The reaction mixture was stirred at 60 °C for 2 h. More EtBr was added until less than 1% of 2- acetamido-5-nitrophenol (**10**) was present, as determined by TLC. An amount of 4 L of H_2O was added to the reaction mixture after it was cooled to 25 °C. The suspension was stirred in H_2O for 30 min, filtered, washed with 4 × 1 L of warm (50 °C) H_2O (maintain pH < 8) and 1 L of heptane, and dried at 60−65 °C in an oven to yield 447.8 g (98%) of product **11**: mp = 164−165 °C.

3-(4-Acetamido-3-ethoxyaniline)-2-cyanopropenoic Acid Ethyl Ester (14). An amount of 410 g (1.83 mol) of 4-acetamido-3-ethoxynitrobenzene (**11**) in 4.2 L THF was reduced to 3-(4-acetamido-3-ethoxyaniline)-2-cyanopropenoic acid ethyl ester under hydrogenation (at 28−30 °C, 50 psi) with 35 g of

10% Pd−C for 3 h. The reaction mixture was filtered through a 0.2 μm cartridge and rinsed with THF. The filtrate was concentrated to give the aniline, which was stirred in 1.3 L of toluene containing 50 g of anhydrous Na_2SO_4 at 25 °C for 20 min. The suspension was filtered, and the cake was washed with 200 mL of toluene. To the filtrate containing **12** in 1.5 L of toluene was added 475 g (2.8 mol) of ethyl (ethoxymethylene)cyanoacetate **13** and 3.5 L of toluene. The suspension was stirred and refluxed at 90 °C for 16 h. The reaction mixture was cooled to room temperature over 1 h and stirred at room temperature for 30 min. The light-yellow solid was filtered, washed with toluene (2 × 400 mL), and dried in a vacuum oven at 60−65 °C for 24 h to give 524 g (90.3%) of **14** as a mixture of *E* and *Z* isomers.

3-Cyano-7-ethoxy-4-hydroxy-6-*N*-acetylquinoline (15). An amount of 210 g (0.664 mol) of **14** was stirred in 12 L of Dowtherm A at 250−255 °C for 20 h. After the mixture was cooled to room temperature, the solid was filtered and washed with toluene (4 × 210 mL). The solid crude material was stirred in THF at reflux temperature (66 °C) for 30 min and cooled. The dark-brown solid was filtered, washed with THF (4 × 100 mL), and dried in a 60−65 °C vacuum oven to yield 75.3 g (42%) of **15**: mp > 250 °C.

4-Chloro-3-cyano-7-ethoxy-6-*N*-acetylquinoline (16). To a dark-brown suspension of 80.0 g (0.296 mol) of **15** in 1.6 L of diethylene glycol dimethyl ether was added 96.0 mL (1.04 mol) of phosphorus oxychloride in one portion. The reaction mixture was stirred at 100−102 °C for 45 min. The reaction mixture was cooled to 80−85 °C, and 25 g of Celite was added. The reaction mixture was filtered through a Celite pad with 3 × 100 mL and 1 × 50 mL of diethylene glycol dimethyl ether. The filtrate was concentrated to a volume of 875 mL and stirred in an aqueous solution of 75.0 g (0.543 mol) of K_2CO_3 (maintaining temperature less than 50 °C). The yellow solid was filtered, washed with 3 × 100 g of warm H_2O and 200 mL of toluene, then dried at 60−65 °C in an oven to yield 55.45 g (65%) of **16**: mp = 250 °C.

***N*-{4-[3-Chloro-4-(1-methyl-1*H*-imidazol-2-ylmethoxy)-phenylamino]-3-cyano-7-ethoxyquinolin-6-yl}acetamide Hydrochloride (18a).** A mixture of 6.10 g (0.0211 mol) of *N*-(4-chloro-3-cyano-7-ethoxyquinolin-6-yl)acetamide (**16**), 4.55 g (0.0191 mol) of 3-chloro-4-(1-methyl-1*H*-imidazol-2-ylmethoxy)phenylamine (**17a**), and 2.21 g (0.0191 mol) of pyridine hydrochloride in 50 mL of *i*-PrOH was refluxed for 16 h. The solid was filtered and washed with H_2O and ether to give 2.86 g of **18a**: mp = 251−252 °C; MS (ES+) *m/z* 491.1 $(M + H)^{+1}$.

***N*-[4-(1-Benzenesulfonyl-1*H*-indol-5-ylamino)-3-cyano-7-ethoxyquinolin-6-yl]acetamide (18c).** A solution of 3.29 g (0.0113 mol) of *N*-(4-chloro-3-cyano-7-ethoxyquinolin-6-yl)-acetamide (**16**), 3.09 g (0.0113 mol) of 1-benzenesulfonyl-1*H*-indol-5-ylamine (**17c**),[34] and 1.31 g (0.0113 mol) of pyridine hydrochloride in 50 mL of 2-methoxyethanol was refluxed for 2 h. The reaction mixture was poured into H_2O. The resulting mixture was extracted with 3 × 250 mL portions of EtOAc. The combined extracts were concentrated in vacuo until a solid formed. This was filtered to give 3.04 g (51%) of the product (**18c**): mp = 255−257 °C; MS (ES+) *m/z* 526.0 $(M + H)^{+1}$.

6-Amino-4-[3-chloro-4-(1-methyl-1*H*-imidazol-2-ylmethoxy)phenylamino]-7-ethoxyquinoline-3-carbonitrile (19a). A mixture of 3.27 g of *N*-{4-[3-chloro-4-(1-methyl-1*H*-imidazol-2-ylmethoxy)phenylamino]-3-cyano-7-ethoxyquinolin-6-yl}acetamide hydrochloride (**18a**) in 10 mL of H_2O and 20 mL of 12 N HCl was stirred and heated in a oil bath for 2 h. The solvents were evaporated in vacuo, and the solid was treated with 50 mL of saturated aqueous $NaHCO_3$, and then filtered. This solid was taken up in EtOAc and passed through Magnesol. This filtrate was evaporated to a solid in vacuo and digested with EtOAc to give 1.77 g (59%) of product (**19a**): mp = 222−225 °C; MS (ES+) *m/z* 449.0 $(M + H)^{+1}$.

6-Amino-4-(1-benzenesulfonyl-1*H*-indol-5-ylamino)-7-ethoxyquinoline-3-carbonitrile (19c). A mixture of 2.82 g (5.27 mmol) of *N*-[4-(1-benzenesulfonyl-1*H*-indol-5-ylamino)-3-cyano-7-ethoxyquinolin-6-yl]acetamide (**18c**) in 20 mL of 12 N HCl and 10 mL of H_2O was stirred and heated in an oil bath at 133 °C for 1 h. When the mixture was cooled, a solid formed. This was filtered and stirred with 100 mL of saturated aqueous $NaHCO_3$. The solid was filtered to give 2.45 g (94%) of the product (**19c**): mp = 263−267 °C; MS (ES+) *m/z* 641.3 $(M + H)^{+1}$.

(*E*)-*N*-(4-{3-Chloro-4-[(1-methyl-1*H*-imidazol-2-yl)-methoxy]anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (21a). The title compound was prepared from 1.18 g (7.13 mmol) of 4-(dimethylamino)-1-yl-but-2-enoic acid, 0.56 mL (820 mg, 6.46 mmol) of oxalyl chloride, and 1.6 g (3.56 mmol) of 6-amino-4-[3-chloro-4-(1-methyl-1*H*-imidazol-2-ylmethoxy)phenylamino]-7-ethoxyquinoline-3-carbonitrile (**19a**) using the method described above for **8d** to yield 1.031 g (52%) of **21a** as a yellow solid: mp = 203−206 °C; MS (ES+) *m/z* 560.1 $(M + H)^{+1}$, 281.3 $(M + H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H,), 9.49 (s, 1H), 8.97 (s, 1H), 8.48 (s, 1H), 7.37 (m, 3H), 7.21 (m, 2H), 6.89 (s, 1H), 6.78 (dt, 1H, J = 15.4 Hz, J = 5.9 Hz), 6.38 (d, 1H, J = 15.4 Hz), 5.24 (s, 2H), 4.31 (q, 2H, J = 6.96 Hz), 3.73 (s, 3H), 3.08 (d, 2H, J = 5.58 Hz), 2.18 (s, 6H), 1.47 (t, 3H, J = 6.96 Hz). Anal. ($C_{29}H_{30}ClN_7O_3 \cdot 0.5H_2O$) C, H, N.

(2*E*)-*N*-{4-[(1-Benzyl-2,3-dihydro-1*H*-indol-5-yl)amino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (21b). The title compound was prepared from 662 mg (4.25 mmol) of 4-(dimethylamino)-1-yl-but-2-enoic acid, 0.35 mL (508 mg, 4.0 mmol) of oxalyl chloride, and 871 mg (2.83 mmol) of 6-amino-4-(1-benzyl-2,3-dihydro-1*H*-indol-5-ylamino)-7-ethoxyquinoline-3-carbonitrile (**19b**) using the method described above for **8d** to yield 585 mg (53%) of a tan solid (**21b**): mp = 199−201 °C; MS (ES+) *m/z* 546.9 $(M + H)^{+1}$, 274.0 $(M + 2H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.49 (s, 1H), 9.46 (s, 1H), 9.19 (s, 1H), 9.46 (s, 1H), 8.91 (s, 1H), 8.35 (s, 1H), 7.32 (m, 7H), 6.97 (s, 1H), 6.92 (dd, 1H, J = 8.28 Hz, J = 1.88 Hz), 6.77 (dt, 1H, J = 5.96 Hz, J = 15.36 Hz), 6.58 (m, 2H), 4.48, (s, 1H), 4.28 (q, 2H, J = 7.04 Hz), 3.29, (t, 2H, J = 6.04 Hz), 2.92 (t, 2H, J = 8.20 Hz) 2.18 (s, 6H), 1.46 (t, 3H, J = 6.92 Hz). Anal. ($C_{33}H_{34}N_6O_2$) C, H, N.

(*E*)-*N*-(3-Cyano-7-ethoxy-4-{[1-(phenylsulfonyl)-1*H*-indol-5-yl]amino}-6-quinolinyl)-4-(dimethylamino)-2-butenamide (21c). The title compound was prepared from 662 mg (4 mmol) of 4-(dimethylamino)-1-yl-but-2-enoic acid, 0.31 mL (457 mg, 3.6 mmol) of oxalyl chloride, and 1.971 g (2 mmol) of 6-amino-4-(1-benzenesulfonyl-1*H*-indol-5-ylamino)-7-ethoxyquinoline-3-carbonitrile (**19c**) using the method described above for **8d** to yield 517 mg (43%) of a tan solid (**21c**): mp = 243−245 °C; MS (ES+) *m/z* 595.0 $(M + H)^{+1}$, 298.2 $(M + H)^{+2}$; ^{1}H NMR (DMSO-d_6) δ 9.69 (s, 1H), 9.48 (s, 1H), 8.98 (s, 1H), 8.45 (s, 1H), 7.95 (m, 3H), 7.76 (d, 1H, J = 3.66 Hz), 7.69 (m, 1H), 7.58 (m, 2H), 7.45 (d, 1H, J = 1.92 Hz), 7.39 (s, 1H), 7.27 (dd, 1H, J = 8.85 Hz, J = 2.04 Hz), 6.84 (d, 1H, J = 3.66 Hz), 6.75 (dt, 1H, J = 15.39 Hz, J = 5.88 Hz), 6.29 (m, 1H), 4.31 (q, 2H, J = 6.84 Hz), 3.07, d, 2H, J = 5.61 Hz), 2.17 (s, 6H), 1.47 (t, 3H, J = 6.93 Hz). Anal. ($C_{32}H_{30}N_6O_4S \cdot 0.25H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(2-chlorobenzyloxy)-phenylamino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (21d). A 958 mg (2 mmol) sample of **19d** was reacted with 4-(dimethylamino)-but-2-enoic acid chloride (**20**) as described above for **8d** to yield 780 mg (66%) of product as a yellow glass after silica gel chromatography: HRMS (ESI) *m/z* 590.171 17 $(M + H)^{+1}$, Δ = −0.86 mmu; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.49 (s, 1H), 8.97 (s, 1H), 8.48 (s, 1H), 7.68−7.65 (m, 1H), 7.55−7.53 (m, 1H), 7.44−7.38 (m, 4H), 7.30−7.27 (m, 1H), 7.22 (dd, 1H, J = 6.6, 1.9 Hz), 6.81−6.74 (m, 1H), 6.61−6.57 (m, 1H), 5.27 (s, 2H), 4.31 (q, 2H, J = 5.2 Hz), 3.07 (d, 2H, J = 3.8 Hz), 2.18 (s, 6H), 1.47 (t, 3H, J = 5.2 Hz). Anal. ($C_{31}H_{29}Cl_2N_5O_3 \cdot 1H_2O$) C, H, N, Cl.

Synthesis Using Method 3. 4-Chloro-6-(5-(dimethylamino)-2-oxopent-3-enyl)-7-ethoxyquinoline-3-carbonitrile (23). A solution of 4-dimethylaminocrotonyl chloride freshly prepared from 9 g (54.3 mmol) of 4-dimethylaminocrotonic acid hydrochloride was slurried in 20 mL of acetonitrile, and 6.69 g (27 mmol) of 6-amino-4-chloro-7-ethoxyquinoline-3-carbonitrile (**22**) as a slurry in 150 mL of 1-methyl-2-pyrrolidinone was added under nitrogen. This was cooled in

an ice bath and stirred for 2.5 h. The solution was poured into 1.3 L of a 1:1 mixture of saturated $KHCO_3$ and water and was stirred for 20 min, and the precipitate was filtered off, washed with water, and dried. Crystallization from acetonitrile yielded 7.97 g (82%) of brown solid in two crops, ESMS *m/z* 358.8 $(M + H)^{+1}$. The product was used without further purification.

(*E*)-*N*-(4-{Chloro-4-[(4-fluorobenzyl)oxy]anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (25g). A mixture of 0.350 g (0.975 mmol) of (*E*)-*N*-(4-chloro-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (**23**), 0.295 g (1.17 mmol) of 3-chloro-4-(4-fluorobenzyloxy)aniline, and 0.226 g (1.95 mmol) of pyridine hydrochloride in 20 mL of *i*-PrOH was refluxed under N_2 for 10.5 h. The cooled reaction mixture was poured into 100 mL of H_2O and basified with K_2CO_3. Addition of EtOAc caused the gummy mixture to solidify, and this material was collected, washed with EtOAc, and dried. A 0.545 g sample of crude product was purified by flash chromatography (silica, 8% MeOH in $CHCl_3$) to give 0.510 g (53%) of yellow-orange foam: HRMS (ESI) *m/z* 574.199 63 $(M + H)^{+1}$, $\Delta = -0.69$ mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.49 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.54 (m, 2H), 7.37 (s, 2H), 7.25 (m, 4H), 6.78 (m, 1H), 6.59 (m, 1H), 5.20 (s, 2H), 4.30 (q, 2H, $J = 6.96$ Hz), 3.07 (d, 2H, $J = 5.52$ Hz), 2.18 (s, 6H), 1.47 (t, 3H, $J = 6.96$ Hz). Anal. ($C_{31}H_{29}ClFN_5O_3 \cdot 0.4H_2O$) C, H, N.

(*E*)-*N*-[4-(3-Chloro-4-phenoxyanilino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamid (25a). This compound was prepared as a yellow solid (0.57 g, 75%) by method 3 described for the preparation of **25g** using **23** and 0.366 g (3.62 mmol) of 3-chloro-4-phenoxyphenylamine: HRMS (ES+) *m/z* 542.195 57 $(M + H)^{+1}$, $\Delta = 0.22$ mmu; ^{1}H NMR (DMSO-d_6) δ 9.78 (s, 1H), 9.51 (s, 1H), 9.02 (s, 1H), 8.55 (s, 1H), 7.46 (s, 1H), 7.42 (s, 1H), 7.35 (t, 2H, $J = 6.3$ Hz), 7.24 (d, 1H, $J = 3.3$ Hz), 7.18 (d, 1H, $J = 6.6$ Hz), 7.09 (t, 1H, $J = 6.6$ Hz), 6.96 (d, 2H, $J = 6.6$ Hz), 6.82−6.76 (m, 1H), 6.62 (d, 1H, $J = 9.9$ Hz), 4.32 (q, 2H, $J = 6.6$ Hz), 3.08 (d, 2H, $J = 3$ Hz), 2.17 (s, 6H), 1.47 (t, 3H, $J = 3$ Hz). Anal. ($C_{30}H_{28}ClN_5O_3 \cdot H_2O$) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25b). The product was prepared by method 3 described for the preparation of **25g** using 0.36 g (1.0 mmol) of **23**, 0.28 g (1.2 mmol) of 4-benzyloxyaniline hydrochloride, 92 mg (0.8 mmol) of pyridine hydrochloride, and 3.0 mL of methoxyethanol at reflux for 30 min. The product was purified by short column chromatography on silica gel with 25:25:2:1 CH_2Cl_2−EtOAc−MeOH−Et_3N to give 487 mg (94%) of amber glass: MS (ES+) *m/z* 521.9 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.56 (s, 1H), 9.49 (s, 1H), 8.96 (s, 1H), 8.41 (s, 1H), 7.48 (m, 2H), 7.38 (m, 3H,), 7.22 (d, 2H, $J = 6.9$ Hz), 7.05 (d, 2H, $J = 6.9$ Hz), 6.77 (dt, 1H, $J = 5.3$, $J = 15.4$ Hz), 6.58 (d, $J = 15.4$ Hz), 5.12 (s, 2H), 4.29 (q, 2H, $J = 6.9$ Hz), 3.08 (d, 2H, $J = 5.3$ Hz), 2.18 (s, 6H), 1.46 (t, 3H, $J = 6.9$ Hz). Anal. ($C_{31}H_{31}N_5O_3 \cdot$MeOH) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(2-phenylethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25c). 25c was prepared from a mixture of 0.250 g (0.696 mmol) of **23**, 0.207 g (0.835 mmol) of 3-chloro-4-phenylethoxyaniline, and 0.161 g (1.39 mmol) of pyridine hydrochloride in 12 mL of *i*-PrOH using the same procedure described for **25g**. A 1.00 g sample of crude product was purified by flash chromatography (silica gel, 8% MeOH in $CHCl_3$) to give 0.700 g (74%) of tan foam: ^{1}H NMR (DMSO-d_6) δ 9.59 (s, 1H), 9.48 (s, 1H), 8.97 (s, 1H), 8.45 (s, 1H), 7.28 (m, 10H), 6.78 (m, 1H), 6.58 (m, 1H), 4.28 (m, 4H), 3.09 (m, 4H), 2.17 (s, 6H), 1.47 (t, 3H, $J = 6.96$ Hz); HRMS (ESI) *m/z* 570.226 63 $(M + H)^{+1}$, $\Delta = -0.17$ mmu. Anal. ($C_{32}H_{32}ClN_5O_3 \cdot 0.5H_2O$) C, H, N.

(*E*)-*N*-[4-(4-Benzyloxy-3,5-dichlorophenylamino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-butenamide (25d). As described in the preparation of **25g**, 448 mg (1.25 mmol) of **23** and 402 mg (1.5 mmol) of 4-benzyloxy-3,5-dichloroaniline in 35 mL of *i*-PrOH were reacted to yield 203 mg (27%) of beige crystals in two crops from CH_3CN: mp = 106−109 °C; HRMS (ESI) *m/z* 590.171 37 $(M + H)^{+1}$, $\Delta = -0.66$ mmu; ^{1}H NMR (DMSO-d_6) δ 9.74 (bs, 1H), 9.50 (s, 1H), 9.00 (s, 1H), 8.62 (s, 1H), 7.55−7.52 (m, 2H), 7.48−7.33 (m, 6H), 6.81−6.60 (m, 2H), 5.01 (s, 2H), 4.33 (q, 2H, $J = 5.2$ Hz), 3.08 (d, 2H, $J = 5.1$ Hz), 2.17 (s, 6H), 1.48 (t, 3H, $J = 5.2$ Hz). Anal. ($C_{31}H_{29}Cl_2N_5O_3 \cdot 0.5H_2O \cdot 0.25$EtOAc) C, H, N.

(*E*)-*N*-{4-[4-(Benzyloxy)-3-cyanoanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25e). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.374 g (1.67 mmol) of 5-amino-2-(benzyloxy)benzonitrile, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 35 mL of *i*-PrOH using the same procedure as described for **25g**. A 680 mg sample of crude product was purified by flash chromatography (silica gel, 7% MeOH in $CHCl_3$) to give 0.560 g (74%) of off-white solid. ^{1}H NMR (DMSO-d_6) δ 9.68 (s, 1H), 9.51 (s, 1H), 8.97 (s, 1H), 8.49 (s, 1H), 7.69 (m, 1H), 7.46 (m, 8H), 6.77 (m, 1H), 6.59 (m, 1H), 5.31 (s, 2H), 4.30 (q, 2H, $J = 6.9$ Hz), 3.07 (d, 2H, $J = 5.55$ Hz), 2.18 (s, 6H), 1.48 (t, 3H, $J = 6.9$ Hz); HRMS (ESI) *m/z* 547.244 18 $(M + H)^{+1}$, $\Delta = -1.04$ mmu. Anal. ($C_{32}H_{30}N_6O_3 \cdot 1.4H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(2-fluorobenzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25f). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.421 g (1.67 mmol) of 3-chloro-4-[(2-fluorobenzyl)oxy]aniline, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 45 mL of *i*-PrOH using the same procedure described for **25g**. A 0.740 g sample of crude product was purified by flash chromatography (silica gel, 7% MeOH in $CHCl_3$) to give 0.590 g (74%) of yellow solid; HRMS (ESI) *m/z* 574.20070 $(M + H)^{+1}$, $\Delta = -0.88$ mmu; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.50 (s, 1H), 8.97 (s, 1H), 8.48 (s, 1H), 7.61 (m, 1H), 7.38 (m, 2H), 7.35 (m, 2H), 7.25 (m, 4H), 6.78 (m, 1H), 6.58 (m, 1H), 5.25 (s, 2H), 4.33 (m, 2H), 3.17 (d, 2H, $J = 3.96$ Hz), 3.07 (d, 2H, $J = 3.84$ Hz), 2.18 (s, 6H), 1.47 (t, 3H, $J = 5.19$ Hz). Anal. ($C_{31}H_{29}ClFN_5O_3 \cdot H_2O$) C, H, N.

(*E*)-*N*-{4-[3,5-Dichloro-4-(3-fluorobenzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25h). A 720 mg (2 mmol) sample of **23** was reacted with 647 mg (2.4 mmol) of 3,5-dichloro-4-[(3-fluorobenzyl)oxy]-aniline as described above for **25g** to yield 457 mg (38%) of beige crystals in two crops from CH_3CN: mp = 128−131 °C; HRMS (ESI) *m/z* 608.162 27 $(M + H)^{+1}$, $\Delta = -0.33$ mmu; ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 9.50 (s, 1H), 8.99 (s, 1H), 8.62 (s, 1H), 7.50−7.41 (m, 2H), 7.38−7.31 (m, 4H), 7.25−7.19 (m, 1H), 6.81−6.74 (m, 1H), 6.64−6.59 (m, 1H), 5.04 (s, 2H), 4.33 (q, 2H, $J = 5.1$ Hz), 3.08 (bs, 2H), 2.17 (s, 6H), 1.48 (t, 3H, $J = 5.1$ Hz). Anal. ($C_{31}H_{28}Cl_2FN_5O_3 \cdot H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(3,5-difluorobenzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25i). As described in the preparation of **25g**, 720 mg (2 mmol) of **23** and 644 mg (2.4 mmol) of 3-chloro-4-(3,5-difluorobenzyloxy)aniline were reacted to yield 432 mg (37%) of golden brown crystals from CH_3CN: mp = 101−104 °C; HRMS (CI) *m/z* 592.1913 $(M + H)^{+1}$, $\Delta = 1.4$ mmu; ^{1}H NMR (DMSO-d_6) δ 9.73 (s, 1H), 9.63 (s, 1H), 8.97 (s, 1H), 8.48 (s, 1H), 7.39−7.37 (m, 2H), 7.25−7.20 (m, 5H), 6.81−6.58 (m, 2H), 5.27 (s, 2H), 4.31 (q, 2H, $J = 5.2$ Hz), 3.08 (d, 2H, $J = 3.9$ Hz), 2.18 (s, 6H), 1.47 (t, 3H, $J = 5.2$ Hz). Anal. ($C_{31}H_{28}ClF_2N_5O_3 \cdot 1.12H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(3-cyanobenzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25k). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.431 g (1.67 mmol) of 3-[(4-amino-2-chlorophenoxy)methyl]benzonitrile, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 36 mL of *i*-PrOH using the same procedure described for **25g**. A 0.740 g sample of crude product was purified by flash chromatography (silica gel, 7% MeOH in $CHCl_3$) to give 0.600 g (74%) of yellow solid. ^{1}H NMR (DMSO-d_6) δ 9.63 (s, 1H), 9.06 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.94 (s, 1H), 7.83 (m, 2H), 7.65 (m, 2H), 7.39 (s, 2H), 7.23 (m, 2H), 6.77 (m, 1H), 6.59 (m, 1H), 5.29 (s, 2H), 4.30 (q, 2H, $J = 5.16$ Hz), 3.07 (d, 2H, $J = 3.9$ Hz), 2.17 (s, J

= 6 Hz), 1.47 (t, 3H, J = 5.16 Hz); HRMS (ESI) m/z 291.106 65 $(M + 2H)^{+2}$, Δ = 0.68 mmu. Anal. ($C_{32}H_{29}ClN_6O_3 \cdot H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(3-methylbenzyloxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25m). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.414 g (1.67 mmol) of 3-chloro-4-(3-methylbenzyloxy)aniline, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 25 mL of *i*-PrOH using the same procedure as for **25g**. A 0.700 g sample of crude product was purified by flash chromatography (silica, 5% MeOH in $CHCl_3$) to give 430 mg (54%) of tan foam: HRMS (ESI) m/z 570.225 10 $(M + H)^{+1}$, Δ = −0.27 mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.49 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.29 (m, 7H), 6.78 (m, 1H), 6.59 (M, 1H), 5.17 (s, 2H), 4.30 (q, 2H, J = 5.19 Hz), 3.07 (d, 2H, J = 3.99 Hz), 2.33 (s, 3H), 2.18 (s, 6H), 1.47 (t, 3H, J = 5.19 Hz). Anal. ($C_{32}H_{32}ClN_5O_3 \cdot 0.6H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(cyclohexylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25n). The title compound was prepared from a mixture of 0.650 g (1.81 mmol) of **23**, 0.521 g (2.17 mmol) of 3-chloro-4-(cyclohexylmethoxy)aniline, and 0.420 g (3.62 mmol) of pyridine hydrochloride in 30 mL of *i*-PrOH using the same procedure as described for **25g**. A 1.00 g sample of crude product was purified by flash chromatography (silica gel, 7% MeOH in $CHCl_3$) to give 0.860 g (84%) of yellow foam: HRMS (ESI) m/z 281.632 08 $(M + 2H)^{+2}$, Δ = −0.38 mmu; ^{1}H NMR (DMSO-d_6) δ 9.59 (s, 1H), 9.49 (s, 1H), 8.96 (s, 1H), 8.46 (s, 1H), 7.38 (s, 1H), 7.33 (d, 1H, J = 1.86 Hz), 7.17 (m, 2H), 6.78 (m, 1H), 5.59 (m, 1H), 4.33 (q, 2H, J = 3.84 Hz), 3.87 (d, 2H, J = 4.62 Hz), 3.07 (d, 2H, J = 3.87 Hz), 2.18 (s, 6H), 1.80 (m, 6H), 1.47 (t, 3H, J = 5.22 Hz), 1.16 (m, 5H). Anal. ($C_{31}H_{36}ClN_5O_3 \cdot 0.25H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25o). This compound was prepared as a yellow solid (0.86 g, 85%) by the method described for **25g** using 0.65 g (1.81 mmol) of **23** and 0.42 g (3.62 mmol) of 3-chloro-4-(2-pyridinylmethoxy)aniline: HRMS (ES+) m/z 557.205 89 $(M + H)^{+1}$, Δ = −0.36 mmu; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 9.49 (s, 1H), 8.96 (s, 1H), 8.60 (d, 1H, J = 3.9 Hz), 8.47 (s, 1H), 7.88 (t, 1H, J = 3.9 Hz), 7.58 (d, 1H, J = 3.9 Hz), 7.39−7.35 (m, 3H), 7.26 (d, 1H, J = 7.8 Hz), 7.19 (d, 1H, J = 8.1 Hz), 6.81−6.73 (m, 1H), 6.59 (d, 1H, J = 7.8 Hz), 5.28 (s, 2H), 4.30 (q, 2H, J = 6.9 Hz), 3.07 (d, 2H, J = 3.9 Hz), 2.17 (s, 6H), 1.46 (t, 3H, J = 3.9 Hz). Anal. ($C_{30}H_{29}ClN_6O_3 \cdot 1.1H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(2-furylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25q). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.374 g (1.67 mmol) of 3-chloro-4-(2-furylmethoxy)aniline, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 25 mL of *i*-PrOH using the same procedure as for **25g**. A 0.600 g sample of the crude product was purified by flash chromatography (silica gel, 5% MeOH in $CHCl_3$) to give 0.360 mg (47%) of brown foam: HRMS (ESI) m/z 546.192 59 $(M + H)^{+1}$, Δ = 2.33 mmu; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.49 (s, 1H), 8.97 (s, 1H), 8.47 (s, 1H), 7.73 (s, 1H), 7.34 (m, 3H), 7.21 (m, 1H), 6.77 (m, 1H), 6.62 (m, 2H), 6.49 (m, 1H), 5.16 (s, 2H), 4.30 (q, 2H, J = 5.19 Hz), 3.07 (d, 2H, J = 3.69 Hz), 2.18 (s, 6H), 1.47 (t, 3H, J = 5.19 Hz). Anal. ($C_{29}H_{28}ClN_5O_4 \cdot 0.3H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(1-naphthylmethoxy)anilino[-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25t). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.474 g (1.67 mmol) of 3-chloro-4-(1-naphthylmethoxy)aniline, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 23 mL of *i*-PrOH using the same procedure as described for **25g**. The crude product was dissolved in $CHCl_3$−MeOH, neutralized with concentrated NH_4OH, and evaporated. A 0.960 mg sample of this material was purified by flash chromatography (silica gel, 7% MeOH in $CHCl_3$) to give 0.720 mg (86%) of brown foam: HRMS (ESI) m/z 606.226 84 $(M + H)^{+1}$, Δ = 0.19 mmu; ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 1H), 9.51 (s, 1H), 8.98 (s, 1H), 8.48 (s, 1H), 8.14 (d, 1H, J = 5.91 Hz), 7.98 (m, 2H), 7.74 (d, 1H, J = 5.01 Hz), 7.56 (m, 3H), 7.46 (d, 2H, J = 6.69 Hz), 7.38 (m, 2H), 7.25 (dd, 1H, J = 6.6 Hz, J = 1.89 Hz), 6.78 (m, 1H), 6.60 (m, 1H), 5.67 (s, 2H), 4.31 (q, 2H, J = 5.16 Hz), 3.07 (d, 2H J = 3.81 Hz), 2.17 (s, 6H), 1.47 (t, 3H, J = 5.16 Hz). Anal. ($C_{35}H_{32}ClN_5O_3 \cdot 0.5H_2O$) C, H, N.

(*E*)-*N*-{4-[3-Chloro-4-(2-naphthylmethoxy)anilino[-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (25u). The title compound was prepared from a mixture of 0.500 g (1.39 mmol) of **23**, 0.474 g (1.67 mmol) of 3-chloro-4-(2-naphthylmethoxy)aniline, and 0.322 g (2.78 mmol) of pyridine hydrochloride in 25 mL of *i*-PrOH using the same procedure as described for **25g**. A 0.900 g sample of crude product was purified by flash chromatography (silica gel, 7% MeOH in $CHCl_3$) to give 0.590 g (70%) of yellow foam: HRMS (ESI) m/z 606.225 88 $(M + H)^{+1}$, Δ = −0.77 mmu; ^{1}H NMR (DMSO-d_6) δ 9.63 (s, 1H), 9.51 (s, 1H), 8.98 (s, 1H), 8.47 (s, 1H), 7.96 (m, 4H), 7.62 (d, 1H, J = 7.53 Hz), 7.54 (m, 2H), 7.39 (m, 2H), 7.30 (m, 1H), 7.23 (m, 1H), 6.78 (m, 1H), 6.59 (m, 1H), 5.39 (s, 2H), 4.30 (q, 2H, J = 5.19 Hz), 3.06 (d, 2H, J = 3.75 Hz), 2.17 (s, 6H), 1.47 (t, 3H, J = 5.19 Hz). Anal. ($C_{35}H_{32}ClN_5O_3$) C, H, N.

(*E*)-*N*-[4-(3-Chloro-4-{[(1*R*)-1-phenylethyl]oxy}anilino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (25v). This compound was prepared as a yellow solid (0.6 g, 74%) by the same method for the preparation of **25g**, using 0.5 g (1.39 mmol) of **23** and 0.414 g (1.67 mmol) of 3-chloro-4-{[(1*R*)-1-phenylethyl]oxy}aniline: MS(ESI+) m/z 570.2 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.54 (s, 1H), 9.47 (s, 1H), 8.93 (s, 1H), 8.44 (s, 1H), 7.43 (d, 2H, J = 6 Hz), 7.36−7.33 (m, 4H), 7.27 (t, 1H, J = 5.1 Hz), 7.05 (t, 2H, J = 3.3 Hz), 6.78−6.72 (m, 1H), 6.58 (d, 1H, J = 9.3 Hz), 5.61 (q, 1H, J = 6 Hz), 4.29 (q, 2H, J = 6.3 Hz), 3.06 (d, 2H, J = 6.3 Hz), 2.16 (s, 6H), 1.58 (d, 3H, J = 3.3 Hz), 1.45 (t, 3H, J = 6.3 Hz). Anal. ($C_{32}H_{32}ClN_5O_3 \cdot 0.5H_2O$) C, H, N.

(*E*)-*N*-[4-(3-Chloro-4-{[(1*S*)-1-phenylethyl]oxy}anilino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (25w). This compound was prepared as a yellow solid (0.56 g, 70%) by the same method described for **25g** using 0.5 g (1.39 mmol) of **23** and 0.414 g of (1.67 mmol) 3-chloro-4-{[(1*S*)-1-phenylethyl]oxy}aniline: HRMS (ESI) m/z 570.225 71 $(M + H)^{+1}$, Δ = −0.94 mmu; ^{1}H NMR (DMSO-d_6) δ 9.55 (s, 1H), 9.48 (s, 1H), 8.93 (s, 1H), 8.45 (s, 1H), 7.43 (d, 2H, J = 6.3 Hz), 7.38−7.24 (m, 5H), 7.06 (d, 2H, J = 1.5 Hz), 6.80−6.71 (m, 1H), 6.58 (d, 1H, J = 15.6 Hz), 5.61 (q, 1H, J = 6.3 Hz), 4.29 (q, 2H, J = 6.3 Hz), 3.06 (d, 2H, J = 6.3 Hz), 2.16 (s, 6H), 1.58 (d, 3H, J = 3.3 Hz), 1.45 (t, 3H, J = 6.3 Hz). Anal. ($C_{32}H_{32}ClN_5O_3 \cdot H_2O$) C, H, N.

***N*,*N*-Bis(*tert*-butoxycarbonyl)-2-chloro-4-nitroaniline (27).** To a stirred solution of 8.63 g (50 mmol) of 2-chloro-4-nitroaniline and 0.30 g (2.5 mmol) of DMAP in 50 mL of THF at 0 °C was added 22.9 g (105 mmol) of $(Boc)_2O$. The resulting purple solution was warmed to 25 °C, stirred for 3 h, and concentrated. The residue was partitioned between EtOAc and 1 M citric acid. The EtOAc layer was washed well with brine, dried, and concentrated to give 19.2 g (100%) of off-white solid: mp = 103−110 °C. Anal. ($C_{16}H_{21}ClN_2O_6$) C, H, N.

***N*^{1},*N*^{1}-Bis(*tert*-butoxycarbonyl)-2-chloro-*p*-phenylenediamine (28).** A stirred mixture of 13.5 g (36.2 mmol) of **27**, 7.09 g (127 mg atom) of iron powder, 14.5 mL (253 mmol) of HOAc, and 181 mL of MeOH was refluxed for 30 min and concentrated to remove MeOH. The residue was stirred with EtOAc and H_2O for 15 min and filtered. The EtOAc layer was washed with aqueous $NaHCO_3$ and H_2O, dried, and concentrated to give 12.4 g (100%) of off-white solid: ^{1}H NMR (DMSO-d_6) δ 7.00 (d, 1H, J = 8.6 Hz), 6.77 (bs, 1H), 6.61 (d, 1H, J = 8.6 Hz), 4.46 (bs, 2H), 1.36 (s, 18H).

(*E*)-*N*-[4-(4-Amino-3-chloroanilino)-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (29). A stirred mixture of 3.99 g (11.1 mmol) of **23**, 4.56 g (13.3 mmol) of **28**, 2.56 g (22.2 mmol) of pyridine hydrochloride, and 67 mL of methoxyethanol was refluxed for 1 h. The cooled mixture was stirred with dilute aqueous K_2CO_3 (pH ∼9). The resulting

precipitate was filtered, washed well with water, and dried to give 5.84 g brown solid.

The solid was stirred with 111 mL of CH_2Cl_2 at 0 °C and treated with 55 mL of TFA. The resulting solution was warmed to 25 °C, stirred for 15 min, diluted with toluene, and concentrated. The residue was partitioned between 20:1 EtOAc−MeOH and aqueous K_2CO_3. The organic layer was washed with brine, dried, and concentrated. Short column chromatography of the residue on silica gel with final elution by 25:5:1 EtOAc−MeOH−Et_3N gave 3.05 g (60%) of amber solid: mp = 222−232 °C (dec); MS (ES+) *m/z* 465.2, 467.2 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.49 (s, 1H), 9.47 (s, 1H), 8.91 (s, 1H), 8.38 (s, 1H), 7.33 (s, 1H), 7.14 (d, 1H, J = 2.3 Hz), 6.98 (dd, 1H, J = 2.3 Hz, J = 8.6 Hz), 6.80 (d, 1H, J = 8.6 Hz), 6.77 (dt, 1H, J = 5.6 Hz, J = 15.4 Hz), 6.57 (d, 1H, J = 15.4 Hz), 5.41 (bs, 2H), 4.28 (q, 2H, J = 7.0 Hz), 3.09 (d, 2H, J = 5.6 Hz), 2.19 (s, 6H), 1.46 (t, 3H, J = 7.0 Hz) Anal. ($C_{24}H_{25}ClN_6O_2 \cdot 0.5H_2O$) C, H, N.

(*E*)-*N*-{4-[4-(Benzylamino)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide (31). To a stirred suspension of 465 mg (1.0 mmol) of **29** in 4.0 mL of DCE were added successively 0.21 g (2.0 mmol) of benzaldehyde, 0.59 g (2.8 mmol) of $NaBH(OAc)_3$, and 0.23 mL (4.0 mmol) of HOAc at 25 °C. After 18 h the reaction mixture was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. The organic layer was washed with water, dried, and concentrated. Short column chromatography of the residue on silica gel with final elution by 25:25:2:1 CH_2Cl_2−EtOAc−MeOH−Et_3N gave 375 mg (68%) of an amber foam: MS (ES+) *m/z* 555.1, 557.1 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.49 (s, 1H), 9.46 (s, 1H), 8.89 (s, 1H), 8.37 (s, 1H), 7.33 (s, 1H) 7.40−7.15 (m, 5H), 7.22 (d, 1H, J = 2.3 Hz), 6.98 (dd, 1H, J = 2.3, J = 8.7 Hz), 6.77 (dt, 1H, J = 5.6 Hz, J = 15.4 Hz), 6.59 (d, J = 15.4 Hz), 6.56 (d, 1H, J = 8.7 Hz), 6.16 (t, J = 6.0 Hz), 4.44 (d, 2H, J = 6.0 Hz), 4.30 (q, 2H, J = 6.9 Hz), 3.12 (d, 2H, J = 5.6 Hz), 2.21 (s, 6H), 1.45 (t, 3H, J = 6.9 Hz). Anal. ($C_{31}H_{31}ClN_6O_2 \cdot 0.5H_2CO_3 \cdot 0.5MeOH$) C, H, N.

(*E*)-*N*-(4-{3-Chloro-4-[(1-naphthylmethyl)amino]anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (32). The product was prepared from 465 mg (1.0 mmol) of **29** and 0.31 g (2.0 mmol) of 1-naphthaldehyde as described above for **31** to give 546 mg (91%) of yellow foam: MS (ES+) *m/z* 604.8, 606.7 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.65 (s, 1H), 9.50 (s, 1H), 8.88 (s, 1H), 8.38 (s, 1H), 8.22 (d, 1H, J = 8.2 Hz), 7.97 (dd, 1H, J = 1.6 Hz, J = 8.2 Hz), 7.82 (m, 1H), 7.56 (m, 2H), 7.44 (d, 1H, J = 1.6 Hz), 7.42 (s, 1H), 7.35 (s, 1H), 7.27 (d, 1H, J = 2.4 Hz), 6.99 (dd, 1H, J = 2.4 Hz, J = 8.7 Hz), 6.78 (dt, 1H, J = 5.6 Hz, J = 15.3 Hz), 6.67 (d, 1H, J = 15.3 Hz), 6.58 (d, 1H, J = 8.7 Hz), 6.18 (t, 1H, J = 5.8 Hz), 4.92 (d, 2H, J = 5.8 Hz), 4.29 (q, 2H, J = 6.9 Hz), 3.08 (d, 2H, J = 5.6 Hz), 2.18 (s, 6H), 1.45 (t, 3H, J = 6.9 Hz). Anal. ($C_{35}H_{33}ClN_6O_2 \cdot 2.5H_2O$) C, H, N.

Di(*tert*-butyl)-2-chloro-4-[(3-cyano-7-methoxy-6-nitro-4-quinolinyl)amino]phenylimidodicarbonate (33). A stirred mixture of 5.27 g (20 mmol) of 4-chloro-7-methoxy-6-nitroquinoline-3-carbonitrile (**1a**), 8.22 g (24 mmol) of **28**, and 100 mL of *i*-PrOH was refluxed for 1 h, cooled, and partitioned between EtOAc and aqueous K_2CO_3. The organic layer was washed with water, dried, and concentrated. The crude product was recrystallized from EtOAc−hexane to give 10.3 g (90%) of yellow solid: mp = 235−240 °C (dec); MS (ES+) *m/z* 570.1, 572.1 $(M + H)^{+1}$.

Di(*tert*-butyl)4-[(6-amino-3-cyano-7-methoxy-4-quinolinyl)amino]-2-chlorophenylimidodicarbonate (34). A stirred mixture of 10.2 g (17.9 mmol) of **33**, 3.5 g (62.6 mg atom) of iron powder, 7.2 mL (125 mmol) of HOAc, and 179 mL of MeOH was refluxed for 2 h, cooled, and concentrated to remove MeOH. The residue was stirred in EtOAc−H_2O and filtered to remove iron salts. The organic layer was washed with H_2O, $NaHCO_3$, and H_2O, dried, and concentrated to give 9.08 g of tan solid, which was sufficiently pure for the next step: MS (ES+) *m/z* 540.1, 542.1 $(M + H)^{+1}$.

(*E*)-*N*-[4-(4-Amino-3-chloroanilino)-3-cyano-7-methoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide (35). The acylation of 7.99 g (14.8 mmol) of **34** with 4.90 g (29.6 mmol) of 4-dimethylaminobut-2-enoic acid hydrochloride in 65 mL of MeCN was carried out using the same procedure described for the preparation of **23** to provide 9.06 g of the intermediate *N*,*N*-bis(*tert*-butoxycarbonyl) derivative. The crude product was subjected to short column chromatography on silica gel with final elution by 25:5:1 EtOAc−MeOH−Et_3N to provide 7.30 g (75%) of amber solid, which was sufficiently pure for conversion to the title compound.

The solid was stirred with 112 mL of CH_2Cl_2 at 0 °C and treated with 56 mL of TFA. The resulting solution was warmed to 25 °C, stirred for 5 min, diluted with toluene, and concentrated. The residue was partitioned between 20:1 EtOAc−MeOH and aqueous K_2CO_3. The organic layer was washed with brine, dried, and concentrated to give 4.89 g (97%) of light-yellow solid that was sufficiently pure for further reactions. Short column chromatography of a portion of the product on SG with final elution by 25:5:1 EtOAc−MeOH−Et_3N gave a yellow solid: mp = 190−200 °C (dec); MS (ES+) *m/z* 451.2, 453.2 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.67 (s, 1H), 9.48 (s, 1H), 8.92 (s, 1H), 8.39 (s, 1H), 7.36 (s, 1H), 7.14 (d, 1H, J = 2.3 Hz), 6.98 (dd, 1H, J = 2.3 Hz, J = 8.6 Hz), 6.80 (d, 1H, J = 8.6 Hz), 6.75 (dt, 1H, J = 5.8 Hz, J = 15.4 Hz), 6.58 (d, 1H, J = 15.4 Hz), 5.41 (bs, 2H), 4.01 (s, 3H), 3.15 (d, 2H, J = 5.8 Hz), 2.24 (s, 6H). Anal. ($C_{23}H_{23}ClN_6O_2$) C, H, N.

(*E*)-*N*-(4-{4-[(Benzoyl)amino]-3-chloroanilino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (36). A stirred solution of 226 mg (0.50 mmol) of **35**, 0.34 g (1.5 mmol) of benzoic anhydride, and 1.5 mL of dimethylacetamide (DMA) was heated at 60 °C for 20 h. The reaction mixture was partitioned between 20:1 CH_2Cl_2−MeOH and aqueous K_2CO_3. The organic layer was washed with H_2O, dried, and concentrated. Short column chromatography of the residue on silica gel with final elution by 25:5:1 EtOAc−MeOH−Et_3N gave 0.20 g (72%) of amber solid: mp = 170−180 °C; MS (ES+) *m/z* 555.3, 557.3 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 10.0 (s, 1H), 9.74 (s, 1H), 9.70 (s, 1H), 9.04 (s, 1H), 8.63 (s, 1H), 8.03 (s, 1H), 7.95 (m, 2H), 7.65−7.40 (m, 6H), 6.79 (dt, 1H, J = 5.7 Hz, J = 15.3 Hz), 6.63 (d, 1H, J = 15.3 Hz), 4.06 (s, 3H), 3.02 (d, 2H, J = 5.7 Hz), 2.20 (s, 6H). Anal. ($C_{30}H_{27}ClN_6O_3$) C, H, N.

(*E*)-*N*-(4-{3-Chloro-4-[(nicotinoyl)amino]anilino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (37). A solution of 226 mg (0.50 mmol) of **35** in 1.5 mL of DMA was treated with 164 mg (0.92 mmol) of nicotinyl chloride hydrochloride and 0.16 mL (0.92 mmol) of diisopropylethylamine. The resulting mixture was stirred at 25 °C for 16 h and at 40 °C for 1 h, treated with aqueous K_2CO_3 at 25 °C, stirred for 30 min, and filtered. The yellow solid was washed with a large volume of H_2O and dried to give 0.26 g (94%) of product: mp = 208−218 = °C (dec); MS (ES+) *m/z* 556.3,558.3 $(M + H)^{+1}$; ^1H NMR (DMSO- d_6) δ 10.3 (s, 3H), 9.76 (s, 1H), 9.70 (s, 1H), 9.16 (s, 1H), 9.05 (s, 1H), 8.78 (m, 1H), 8.63 (s, 1H), 8.34 (m, 1H), 7.60−7.10 (m, 5H), 6.79 (dt, 1H, J = 5.6, J = 15.6 Hz), 6.63 (d, 1H, J = 15.6 Hz), 4.05 (s, 3H), 3.07 (d, 2H, J = 5.6 Hz), 2.18 (s, 6H) Anal. ($C_{29}H_{26}ClN_7O_3$) C, H, N.

(*E*)-*N*-(4-{4-[(Anilinocarbonyl)amino]-3-chloroanilino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide (38). A solution of 226 mg (0.50 mmol) of **35** and 0.18 g (1.5 mmol) of phenyl isocyanate in 1.5 mL of DMA was stirred at 25 °C for 60 min, diluted with 20 mL of Et_2O and a few drops of H_2O, and stirred for 30 m. The resulting yellow solid was filtered off, washed with boiling Et_2O, and dried to give 220 mg (77%) of product: mp = 200−210 °C (dec); MS (ES+) *m/z* 570.2, 572.2 $(M + H)^{+1}$; ^1H NMR (DMSO-d_6) δ 9.67 (s, 3H), 9.39 (s, 1H), 8.99 (s, 1H), 8.53 (s, 1H), 8.33 (s, 1H), 8.18 (d, 1H, J = 8.9 Hz), 7.50−7.15 (m, 7H), 6.78 (dt, 1H, J = 5.7 Hz, J = 15.3 Hz), 6.64 (d, 1H, J = 15.3 Hz), 4.04 (s, 3H), 3.08 (d, 2H, J = 5.7 Hz), 2.18 (s, 6H). Anal. ($C_{30}H_{28}ClN_7O_3$) C, H, N.

***N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}ethylenesulfonamide (82).** To a solution of 300 mg (0.68 mmol) of **72** and 0.34 mL (2.43 mmol) of

triethylamine in 34 mL of THF at 0 °C was added 0.212 mL (2.03 mmol) of 2-chloro-1-ethanesulfonyl chloride (**78**) over 10 min. The reaction mixture was stirred at 0 °C for 40 min. Solvent was evaporated. EtOAc, NaOH, and brine were added, and the solid was filtered. After the filtrate was evaporated, acetone was added to dissolve the product. It was filtered, and the acetone solution was evaporated to the crude product as a yellow gum. It was treated with EtOAc and filtered. The EtOAc layer was evaporated and the product was precipitated out with hexane and EtOAc to yield 109 mg (30%) of yellow glass: mp = 96−97 °C; HRMS (ESI) *m/z* 534.119 23, Δ = 0.68 mmu $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 9.69 (s, 1H), 9.5 (bs, 1H), 8.48 (s, 1H), 8.24 (s, 1H), 7.49 (d, 2H, *J* = 7 Hz), 7.44−7.32 (m, 5H), 7.28−7.21 (m, 2H), 6.83 (dd, 1H, *J* = 9.9 Hz, *J* = 16.4 Hz), 6.01−5.91 (m, 2H), 5.22 (s, 2H), 4.23 (q, 2H, *J* = 6.9 Hz), 1.44 (t, 3H, *J* = 6.8 Hz). Anal. ($C_{27}H_{23}ClN_4O_4S\cdot 1.2\ H_2O$) C, H, N.

***N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-2-[(dimethylamino)methyl]acrylamide (83).** An amount of 400 mg (0.9 mmol) of **72** in 4 mL (0.22 mmol) of NMP was cooled to −15 °C. To a solution of 0.298 g (1.80 mmol) of 2-dimethylaminomethylacrylic acid hydrochloride[35] in 15 mL of CH_3CN at 60 °C was added 0.285 mL (3.24 mmol) of $(COCl)_2$ for 30 min. The resulting 2-dimethylaminomethylacrylic acid chloride solution was evaporated to a yellow oil. It was suspended in 15 mL of CH_3CN and added dropwise to an NMP solution (4 mL) containing 400 mg (0.90 mmol) of **72** at −15 °C. The yellow suspension was stirred overnight from −15 °C to room temperature. The reaction mixture was poured into EtOAc−1 N NaOH solution and stirred for 30 min. The resulting yellow precipitate was collected and purified on preparative TLC plates, eluting with 10% MeOH−EtOAc in 1% HOAc to yield 50.5 mg (12%) of light-yellow solid: mp = 110−111 °C; ^{1}H NMR (DMSO-d_6) δ 11.9 (s, 1H), 9.61 (s, 1H), 9.25 (s, 1H), 8.47 (s, 1H), 7.49 (d, 2H, *J* = 7.1 Hz), 7.44−7.35 (m, 6H), 7.26−7.18 (m, 3H), 6.19 (s, 1H), 5.65 (s, 1H), 5.21 (s, 2H), 4.31 (q, 2H, *J* = 6.9 Hz), 2.3 (s, 6H), 1.48 (t, 3H, *J* = 6.9 Hz). Anal. ($C_{31}H_{30}ClN_5O_3\cdot 1.3\ H_2O$) C, H, N.

***N*-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-2-(morpholinylmethyl)acrylamide (84).** To a solution of 281 mg (1.35 mmol) of 2-(morpholin-4yl)methylacrylic acid hydrochloride[35] in 3 mL of CH_3CN at 60 °C was added 0.106 mL (1.22 mmol) of $(COCl)_2$. This was stirred at 60 °C for 15 min, evaporated, and cooled to 0 °C. It was added to a solution of 300 mg (0.68 mmol) of **72** in 3 mL of NMP at 0 °C, and the mixture was stirred overnight from 0 °C to room temperature. The reaction mixture was stirred with saturated $NaHCO_3$. The bright-yellow suspension was filtered and purified by preparative TLC plates developing with 5% MeOH−EtOAc to yield 119 mg (30%) of light-yellow solid: mp = 95−97 °C; HRMS (ESI) *m/z* 597.2144, Δ = 1.37 mmu $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 11.1 (s, 1H), 9.63 (s, 1H), 9.17 (s, 1H), 9.06 (s, 1H), 8.48 (s, 1H), 7.5−7.35 (m, 7H), 7.26−7.17 (m, 2H), 6.24 (d, 1H, *J* = 1.7 Hz), 5.7 (s, 1H), 5.21 (s, 2H), 4.44 (q, 2H, *J* = 6.9 Hz), 3.69 (s, 4H), 3.38 (s, 2H), 2.49 (s, 4H), 1.43 (t, 3H, *J* = 6.9 Hz). Anal. ($C_{33}\ H_{32}ClN_5O_4\cdot 0.5H_2O$) C, H, N.

2-[({4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}amino)methyl]acrylic Acid (85). A 0.8 g (1.8 mmol) amount of **72** in 70 mL of CH_3CN was heated until solids dissolved. To the stirred solution was added 0.37 g (2.2 mmol) of 3-bromo-2-oxopropionic acid and 0.28 g (2.2 mmol) of Hunig's base. The mixture was refluxed for 30 min. Then about 45 mL of the solvent was distilled off. The residue was stirring in H_2O containing 1 mL of Et_3N. The solution was made acidic with acetic acid. The resulting solid was collected and air-dried. The product was purified by chromatography on silica gel, eluting with EtOAc−MeOH (3:1). The product fractions were combined, dissolved in hot EtOH−MeOH, concentrated, and diluted with EtOAc. The product, 0.35 g (37%), was collected. MS (ES+) *m/z* 528.7 $(M + H)^{+1}$; ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H), 7.62 (s, 1H), 7.48 (d, 2H, *J* = 3 Hz), 7.41 (t, 2H, *J* = 6 Hz), 7.34 (m, 1H), 7.08 (d, 1H, *J* = 6.5 Hz), 6.85 (m, 2H), 6.69 (dd, 1H, *J* = 1.9 Hz, *J* = 6.5 Hz), 6.19 (s, 1H), 5.57 (s, 1H), 5.18 (s, 1H), 5.11 (s, 2H), 5.00 (s, 2H), 4.10 (q, 2H, *J* = 5.2 Hz), 3.33 (bs, 2H), 1.37 (t, 3H, *J* = 5.2 Hz). Anal. ($C_{29}H_{25}ClN_4O_4\cdot 1.2H_2O$) C, H, N.

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Supporting Information Available: Experimental procedures for compounds **2d**,**k**, **7a**,**c**,**e**,**g**,**j**−**l**,**n**,**o**,**r**−**u**, **8a**,**c**,**k**,**m**, **17a**, and **25j**,**l**,**p**,**r**,**s** and elementary analysis data for compounds **7a**−**v**, **8a**−**u**, **21a**−**d**, **25a**−**w**, **31**, **32**, **36**−**38**, **75**−**77**, and **82**−**86**. This material is available free of charge via the Internet at http://pubs.acs.org.

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