6-Substituted-4-(3-bromophenylamino)quinazolines as Putative Irreversible Inhibitors of the Epidermal Growth Factor Receptor (EGFR) and Human Epidermal Growth Factor Receptor (HER-2) Tyrosine Kinases with Enhanced Antitumor Activity

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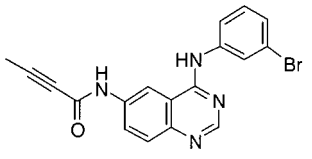
A series of new 6-substituted-4-(3-bromophenylamino)quinazoline derivatives that may function as irreversible inhibitors of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2) tyrosine kinases have been prepared. These inhibitors have, at the C-6 position, butynamide, crotonamide, and methacrylamide Michael acceptors bearing water-solublilizing substituents. These compounds were prepared by acylation of 6-amino-4-(3-bromophenylamino)quinazoline with unsaturated acid chlorides or mixed anhydrides. We show that attaching a basic functional group onto the Michael acceptor results in greater reactivity, due to intramolecular catalysis of the Michael addition and/or an inductive effect of the protonated basic group. This, along with improved water solubility, results in compounds with enhanced biological properties. We present molecular modeling and experimental evidence that these inhibitors interact covalently with the target enzymes. One compound, **16a**, was shown to have excellent oral activity in a human epidermoid carcinoma (A431) xenograft model in nude mice.

Introduction

Receptor protein tyrosine kinases play a key role in signal transduction pathways that regulate cell division and differentiation. Overexpression of certain growth factor receptor kinases such as epidermal growth factor receptor (EGFR) as well as the related human epidermal growth factor receptor (HER-2, also known as *erb*B-2) are markers for poor prognosis in many human cancers.[1,2] Compounds that inhibit the kinase activity of EGFR and/or HER-2 after binding of its cognate ligand are of potential interest as new therapeutic antitumor agents.[3,4]

The 6,7-dialkoxy-4-(phenylamino)quinazolines are a class of potent, selective, ATP-competitive inhibitors of EGFR tyrosine kinase.[5−7] However, in an in vivo environment, such reversible ATP-competitive inhibitors need to compete with a high endogenous ATP concentration (millimolar) within the cell for an extended time in order to exert effective antitumor activity. We[8] and others,[9] therefore, developed irreversible inhibitors based on the 4-(phenylamino)quinazoline core structure that have an attached Michael acceptor functional group at the C-6 or C-7 position. Molecular modeling studies suggested, and experimental results confirmed, that the Michael acceptor side chain of these inhibitors forms a covalent linkage with the sulfhydryl group of the Cys 773 of EGFR. These compounds proved to be potent inhibitors of tumor growth in a human epidermoid carcinoma xenograft (A431) model that overexpresses EGFR. Our irreversible inhibitor, the butynamide-substituted 4-anilinoquinazoline **1**,[10] as well as the acrylamide-substituted 4-anilinoquinazoline **2**, developed independently by us[8] and by the Parke-Davis group,[9] are effective inhibitors of tumor growth. However, these compounds exhibit poor bioavailability characterized by low circulating blood levels after oral administration, primarily because of their low solubility under physiological conditions.



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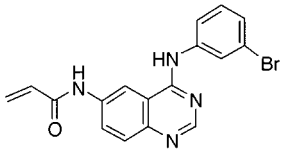

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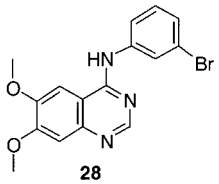

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To improve the pharmacokinetic properties of these compounds, we and others synthesized a series of 4-(phenylamino)quinazolines incorporating water-solubilizing groups. As discussed below, molecular modeling suggests that a favorable site to attach such groups would be at the C-6 and/or C-7 positions, since these positions point toward the outside of the protein. The Parke-Davis group[11] has attached a water-solublizing group at the C-7 position and reserved the C-6 position for the Michael acceptor, usually an acrylamide. In this report we present our strategy, which locates the water-solublizing group directly on the Michael acceptor attached at the C-6 position. Our approach has the

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additional key feature that when the water-solublizing group is a dialkylamino group, it can serve as an intramolecular catalyst for the Michael addition of the sulfhydryl group of Cys 773 via a cyclic catalytic mechanism. Furthermore, under physiological conditions, since the dialkylamino group exists predominantly in its protonated form, it could exert an inductive effect, accelerating the Michael addition. This allows us to modulate the reactivity of our inhibitors and thereby influence their biological activity. This strategy results in a putative irreversible inhibitor, (2*E*)-*N*-{4-[(3-bromophenyl)amino]-6-quinazolinyl}-4-(dimethylamino)-2-butenamide **16a**, that shows superior oral antitumor activity compared to our earlier irreversible inhibitor *N*-{4-[(3-bromophenyl)amino]-6-quinazolinyl}-2-butynamide, **1**, when tested in nude mice bearing the A431 human epidermoid carcinoma.

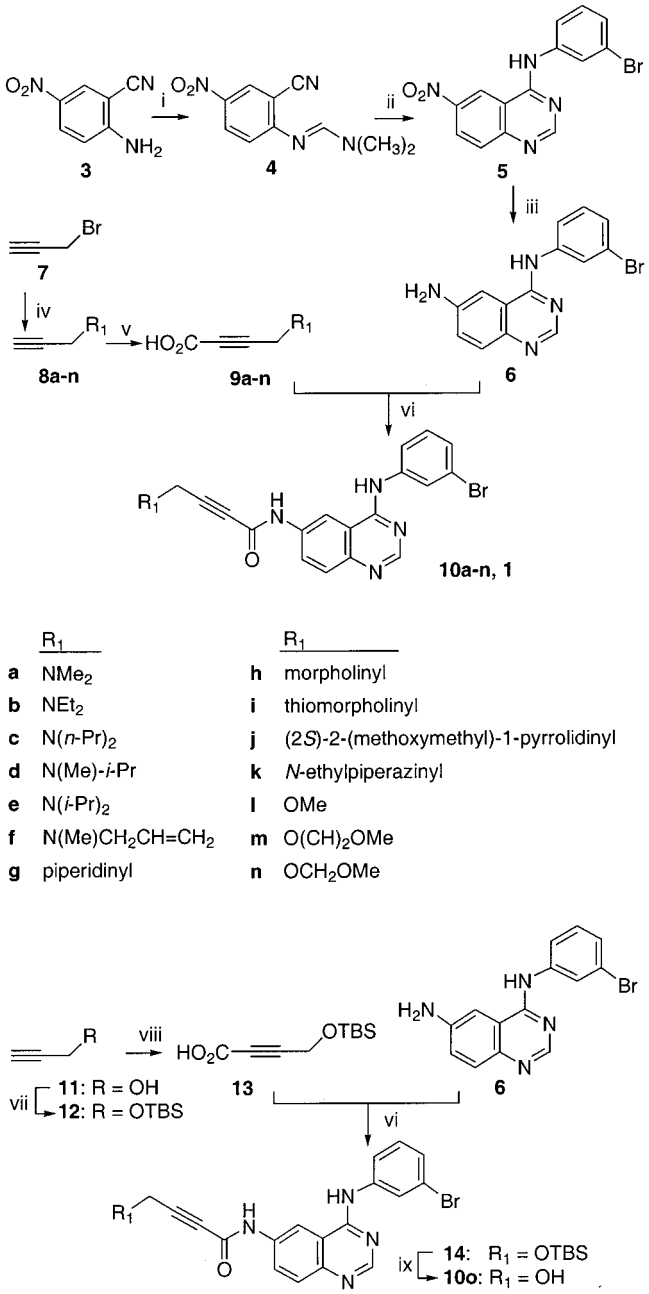
Chemistry

Other workers have prepared 4-anilinoquinazolines by reacting substituted anilines with 4-chloroquinazolines. The 4-chloroquinazolines were prepared by chlorination of the corresponding quinazolinones which, in turn, were prepared by heating anthranilamides with formic acid[12] or triethyl orthoformate[13] or by heating anthranilates with formamide.[14] We designed a more efficient synthesis that allowed ring cyclization and incorporation of the 4-anilino group in a single step, as shown in Scheme 1. Commercially available 5-nitroanthranilonitrile **3** was converted into the corresponding formamidine **4** using DMF acetal. Heating a solution of formamidine **4** and 3-bromoaniline in HOAc gave 6-nitro-4-(3-bromophenylamino)quinazoline **5**. Reduction of the nitro group of **5** with iron in HOAc yielded the intermediate 6-aminoquinazoline **6**. Coupling of **6** with various 4-dialkylamino butynoic acids, **9a**−**k**, or 4-alkoxy butynoic acids, **9l**−**n**, and **13**, was accomplished via a mixed anhydride method.

The propargyl derivatives **8a**−**n** were prepared from propargyl bromide by the displacement with a secondary amine in the presence of cesium carbonate or with a sodium alkoxide prepared from the corresponding alcohol and NaH. Subsequent lithiation and carboxylation yielded the desired 4-substituted butynoic acids **9a**−**n**. For the preparation of compound **10o,** protection of proparyl alcohol **11** with an O-TBS group, followed by lithiation and carboxylation, as before, gave compound **13.** Condensation of **13** with **6** via the mixed anhydride gave **14**, which was subsequently deprotected to give the hydroxy derivative **10o**.

The compounds in the crotonamide series **16a**−**c**, **16g**−**j**, and **16p**−**t** were prepared as shown in Scheme 2. Condensation of **6** with 4-bromocrotonyl chloride gave 4-bromocrotonamide **15**, which was contaminated with the corresponding γ-chloro derivative (4:1 ratio). The chloride presumably came from reaction of the bromide with byproduct HCl. Nucleophilic displacement with various secondary amines yielded 4-substituted crotonamides **16a**−**c**, **16g**−**j**, and **16p**−**t**. The chloride reacted more slowly than the bromide, but nevertheless, the reaction could be driven to completion. The 4-bromocrotonic acid was prepared using Braun's procedure[15] by hydrolysis of methyl 4-bromocrotonate with barium hydroxide. Alternatively, crotonic acid was converted to trimethylsilyl 4-bromocrotonate[16] and then treated with oxalyl chloride to yield 4-bromocrotonyl chloride. Compounds **2**, **17**, and **18** were synthesized by treatment of **6** with the corresponding commercially available acyl chlorides, whereas compounds **19** and **20** were prepared by reacting **6** with 4-methoxycrotonyl chloride[17] and *N*-methyl-1,2,5,6-tetrahydronicotinyl chloride hydrochloride,[18] respectively. 2-(4-Morpholinylmethyl)acrylamide **23** was prepared by coupling **6** with the mixed anhydride **22**, which was made from 2-(4-morpholinylmethyl)acrylic acid **21,**[19] as shown in Scheme 3. 3-Substituted acrylamides **25a** and **25h** were prepared analogously using *trans*-3-chloroacryloyl chloride, followed by condensation with dimethylamine and morpholine, re-

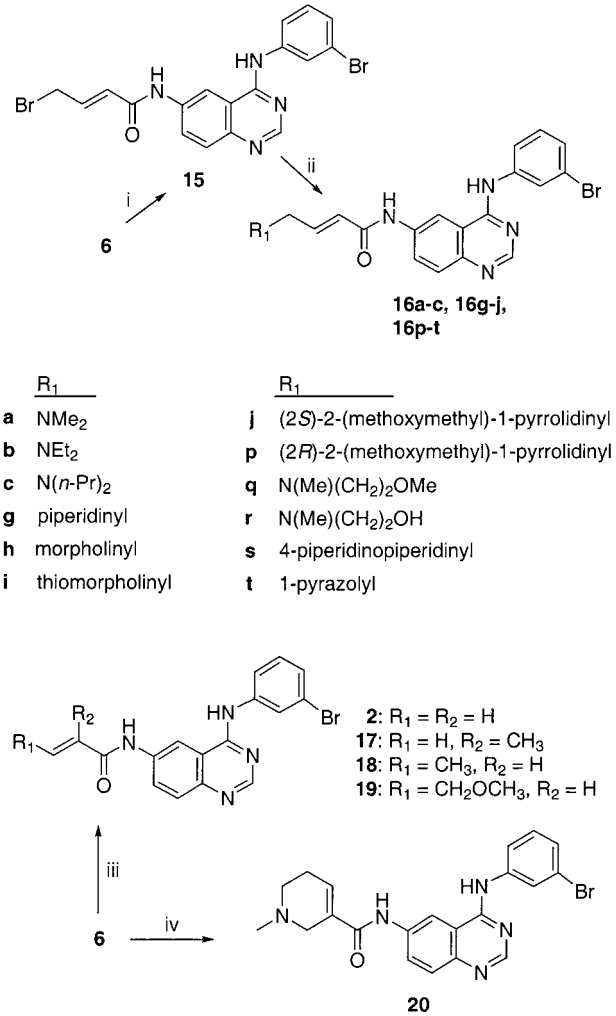
Scheme 1[a]



	R_1		R_1
a	NMe_2	**h**	morpholinyl
b	NEt_2	**i**	thiomorpholinyl
c	$N(n\text{-}Pr)_2$	**j**	(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl
d	N(Me)-*i*-Pr	**k**	*N*-ethylpiperazinyl
e	$N(i\text{-}Pr)_2$	**l**	OMe
f	$N(Me)CH_2CH{=}CH_2$	**m**	$O(CH)_2OMe$
g	piperidinyl	**n**	OCH_2OMe

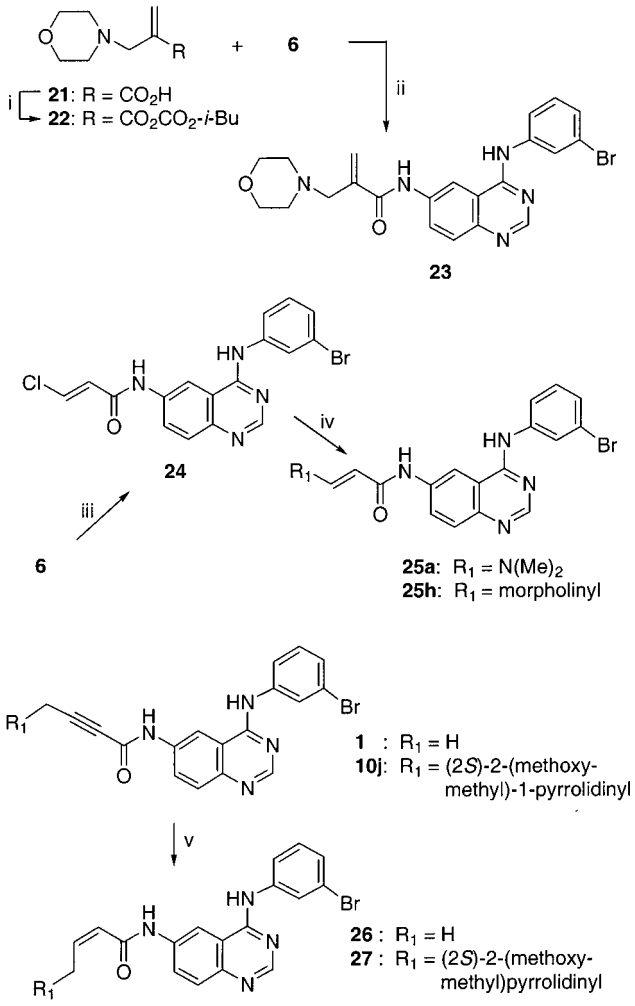
[a] (i) DMF acetal, 100 °C; (ii) 3-bromoaniline, HOAc, reflux; (iii) Fe, HOAc, EtOH, reflux; (iv) R_1H, Cs_2CO_3, acetone, 0 °C (for **8a**−**k**); R_1H, NaH, THF, 0 °C (for **8l**−**n**); (v) *n*-BuLi, THF, then CO_2, −78 °C; (vi) $ClCO_2$-*i*-Bu, *N*-methylmorpholine, THF, 0 °C; (vii) TBSCl, Et_3N, *N*,*N*-(dimethylamino)pyridine, CH_2Cl_2, 25 °C; (viii) MeMgBr, THF, 0 °C, then CO_2; (ix) HOAc, H_2O, THF.

Scheme 2[a]



[a] (i) 4-bromocrotonyl chloride, *N*-methylmorpholine, THF, 0 °C; (ii) R_1H, THF or DMF, 0 °C; (iii) acryloyl chloride, ether, pyridine, 0 °C (for **2**); methacryloyl chloride, ether pyridine 0 °C (for **17**); *trans*-crotonyl chloride, CH_3CN, *N*-methylpyrrolidone, pyridine, 0 °C (for **18**); 4-methoxycrotonyl chloride, THF, *N*,*N*-diisopropylethylamine, 0 °C (for **19**); (iv) *N*-methyl-1,2,5,6-tetrahydronicotinyl chloride hydrochloride, THF, *N*,*N*-diisopropylethylamine, 0 °C.

Scheme 3[a]



[a] (i) $ClCO_2$-*i*-Bu, *N*-methylmorpholine, THF, 0 °C; (ii) THF, pyridine, 0 °C; (iii) *trans*-3-chloroacryloyl chloride, *N*,*N*-diisopropylethylamine, THF, 0 °C; (iv) R_1H, THF, 0 °C; (v) H_2, Lindlar catalyst, MeOH, 25 °C.

spectively. *cis*-Butenamides **26** and **27** were prepared by hydrogenation of the corresponding butynamides **1** and **10j** using Lindlar catalyst.

Molecular Modeling. A homology model for the catalytic domain of EGFR kinase was described in detail in a previous publication from our laboratory.[20] It was developed using a combination of two closely related crystal structures as templates, FGF receptor-1 for the N-terminal lobe and hematopoietic cell kinase (HcK) for the C-terminal lobe. We used the same model when docking quinazoline inhibitor **16a** into the ATP binding site of EGFR kinase. A 7.5 Å sphere of water molecules was added around the ligand and a short (3ps) dynamics run was carried out, followed by several cycles of minimization using Quanta/CHARMm. The entire protein−ligand−water complex was allowed to move during these calculations.

In the final model with quinazoline **16a** (Figure 1), the N1 atom of the quinazoline forms a hydrogen bond with the backbone NH of Met 769, and the N3 atom forms a hydrogen bond to a water molecule. This water molecule, in turn, forms a hydrogen bond with the hydroxyl group of Thr 830. The C2 atom of the quinazoline is 3.87 Å from the backbone carbonyl oxygen of Gln 767, and the C8 atom is 3.12 Å from the backbone carbonyl oxygen of Met 769. The bromoaniline residue lies in a hydrophobic pocket surrounded by Val 702, Ala 719, and Thr 766. Most significantly, the β-carbon atom of the Michael acceptor side chain of quinazoline **16a** is positioned at 4.7 Å from the sulfhydryl group of Cys 773 and is easily accessible for covalent interaction. In addition, this model indicates that the basic side chain of Arg 817 is close to the sulfhydryl hydrogen (3.79 Å); it is possible that this basic side chain might function as a general base catalyst for the Michael addition reaction, particularly for those inhibitors such as **1** and **2,** which lack internal basic functionality. Furthermore, the nitrogen atom of the dimethylamino group on **16a** is situated at 4.64 Å away from the sulfhydryl hydrogen of Cys 773. We are proposing, and present experimental evidence in support below, that this dimethylamino group serves as an intramolecular catalyst for Michael additions to this inhibitor and it functions in this manner after **16a** binds at the active site of EGFR or HER2. Finally, this binding model indicates that the water-solubilizing dimethylamino group points out of the ATP binding pocket toward the solvent environ-

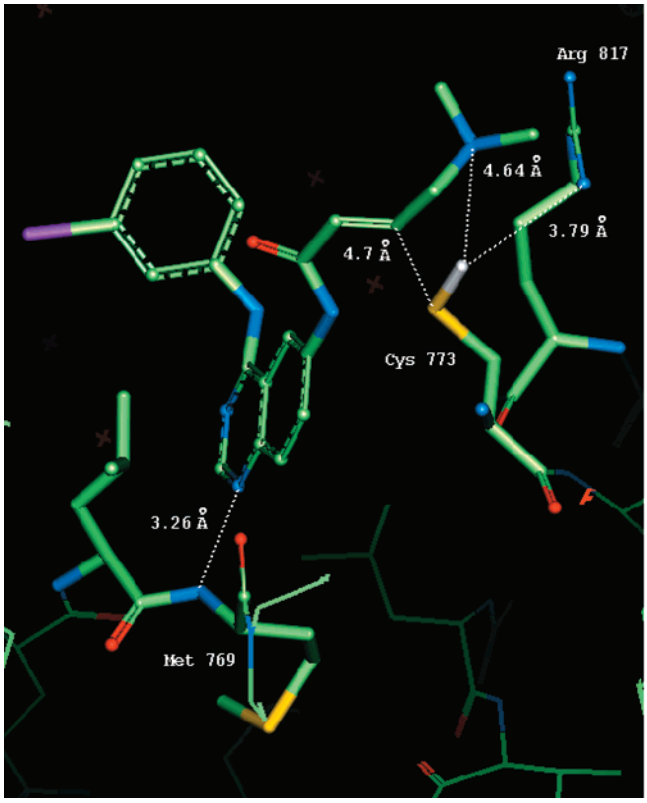


Figure 1. Proposed binding model for quinazoline **16a** built using the homology model for EGFR kinase. Key distances are shown in the figure and discussed in detail in the text.

ment, justifying the decision to place it at the end of the Michael acceptor.

Since EGFR and HER-2 kinases have a high degree of sequence homology in their catalytic domains[21,22] and since Cys 773 is conserved in HER-2 (numbered Cys 805), it is likely that our inhibitors bind in a manner similar to the latter enzyme.

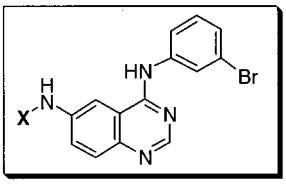
Competitive Reactions with Reduced Glutathione. Since we are proposing that some of our inhibitors function by forming a covalent linkage to Cys 773 of EGFR (and Cys 805 of HER-2) and since the reactivity of these inhibitors might have some bearing on their biological activity, we performed competitive reactivity studies with pairs of these inhibitors using the tripeptide glutathione as a surrogate for the enzymes (see Table 2). In each reaction solution, consisting of a THF−H_2O−CH_3OH mixture, two inhibitors (compounds A and B at 2.5 mM each) were allowed to compete with a limiting quantity of reduced glutathione (1.25 mM). Each evaluation was conducted for 24 h at ambient temperature, unless stated otherwise in the table. In some cases, to observe any Michael addition, it was necessary to add an excess of an external base catalyst. The glutathione conjugates were detected by LC/MS, and the area percents of the conjugate and unreacted inhibitor were measured without calibration, since our emphasis was on the relative reactivity between pairs of compounds toward glutathione. Extinction coefficients for the glutathione conjugates were not determined, since each conjugate has the same chromophore. The results in Table 2 are expressed as the percentage conversion to the glutathione Michael adduct, with 50% being the maximum conversion possible.

Consider the pair of compounds **16a** and **18** in this experiment where no external base catalyst was added. The two inhibitors differ only by the presence of the dimethylamino group on **16a**. Both compounds see exactly the same concentrations of the dimethylamino base catalyst and glutathione in solution, yet a Michael addition occurs only to **16a**, as determined within the limits of detection of our analytical method. Evidently, **16a** is much more reactive than **18**. A similar result is obtained for the pair of compounds **23** and **17**. A reasonable explanation for these observations is that base catalysis for the Michael addition to **16a** and **23** is intramolecular, occurring through cyclic five- and six-membered catalytic mechanisms, respectively. Representations of the transition states are shown in Scheme 4. Another factor that may increase reactivity of **16a** and **23** verses the neutral counterparts **18** and **17**, respectively, is that under the reaction conditions (or under physiological conditions), the amino groups of **16a** and **23** will be protonated to a significant extent. This could result in an increase of the electrophilicity of the double bonds via an inductive effect. Each of these factors, alone or in combination, could account for the increased reactivity of **16a** and **23**. For **16a** we observed a product with the mass corresponding to the simple Michael adduct **29**, while for **23** we detected a product with a mass of 668.7 *m*/*z*, indicating a loss of the morpholine moiety from the initial Michael adduct. On the basis of our HPLC−NMR−MS study of the crude product, we were able to determine the structure of the latter adduct as **31**. This proves that adduct **31** is the primary product of the reaction, not the result of a morpholine elimination from the initial adduct **30** due to a subsequent ionization process in the mass spectrometer. All other products from these reactivity studies showed a mass corresponding to the expected mass of the simple Michael adduct.

Given the arrangement of functionality predicted by our binding model of **16a**, it is reasonable to expect that this type of intramolecular general base catalysis will also operate when our inhibitors bind at the active sites in EGFR and HER-2. In addition, one might expect that the relative reactivity of these pairs of compounds would correlate to some extent with the IC_{50} values we measured for enzyme inhibition.

Other useful information was obtained in these reactivity studies. It is apparent that compounds **1**, **17**, **18**, and **19**, which lack a dialkylamino or morpholine group, either do not react or react extremely slowly with reduced glutathione in the absence of an external base catalyst. The acrylamide **2** is somewhat more reactive, since we did observe the formation of some glutathione conjugate in competition with **16a**. For the compounds that lack a water-solublizing group, the order of reactivity is **2** > **1** > **17**, **18**, **26**. The configuration of the double bond does not seem to be important (compare **18** and **26**). The inhibitor **19,** containing a terminal methoxy group, is far less reactive than **16a**, indicating that it is the basicity of the heteroatom and not simply its electronegativity that is the determining factor. A dimethylamino group is a better intramolecular catalyst than a morpholino group (compare **16a** and **16h**). The methacrylamide-type Michael acceptors are more reactive than the corresponding crotonamides (compare the

Table 1. Inhibition (IC_{50}) of EGFR and HER-2 Kinases and Inhibition (IC_{50}) of Cell Proliferation



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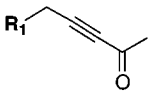

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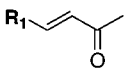

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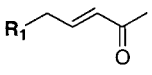

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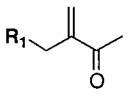

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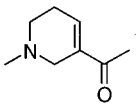

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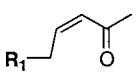


VI

compd	series	R_1	enzyme assays EGFR[a]	enzyme assays HER-2[b]	cell-based assays A431[c]	cell-based assays SKBR3[c]	cell-based assays SW620[c]
1	I	H	0.008	0.378	0.079	0.017	1.500
2	II	H	0.002	0.114	0.095	0.015	4.133
10a	I	NMe_2	0.006	0.014	0.047	0.011	0.530
10b	I	NEt_2	0.012	0.038	0.111	0.006	0.610
10c	I	N(*n*-Pr)$_2$	0.062	0.177	0.042	0.002	0.198
10d	I	N(Me)-*i*-Pr	0.085	0.113	0.077	0.004	0.232
10e	I	N(*i*-Pr)$_2$	0.097	0.162	0.094	0.004	0.302
10f	I	$N(Me)CH_2CH{=}CH_2$	0.071	0.085	0.033	0.001	0.289
10g	I	piperidinyl	0.057	0.073	0.032	0.002	0.302
10h	I	morpholinyl	0.020	0.053	0.150	0.002	1.663
10i	I	thiomorpholinyl	0.095	0.124	0.083	0.002	0.581
10j	I	(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl	0.065	0.117	0.081	0.002	0.324
10k	I	*N*-ethylpiperazinyl	0.065	0.183	0.598	0.062	4.600
10l	I	OMe	0.009	0.033	0.268	0.046	1.730
10m	I	O(CH2)$_2$OMe	0.065	0.116	0.077	0.009	0.945
10n	I	OCH_2OMe	0.059	0.122	0.306	0.092	2.080
10o	I	OH	0.002	0.016	0.315	0.043	1.400
16a	III	NMe_2	0.011	0.301	0.094	0.002	2.336
16b	III	NEt_2	0.073	1.164	0.194	0.002	2.387
16c	III	N(*n*-Pr)$_2$	0.090	1.428	0.145	0.002	2.282
16g	III	piperidinyl	0.106	1.559	0.182	0.004	2.680
16h	III	morpholinyl	0.074	2.776	1.891	0.013	10.68
16i	III	thiomorpholinyl	0.203	2.808	0.589	0.012	8.470
16j	III	(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl	0.131	1.912	0.222	0.002	2.620
16p	III	(2*R*)-2-(methoxymethyl)-1-pyrrolidinyl	0.085	0.733	0.616	0.001	10.100
16q	III	$N(Me)(CH_2)_2OMe$	0.062	0.634	0.564	0.003	5.890
16r	III	$N(Me)(CH_2)_2OH$	0.014	0.414	0.329	0.003	7.530
16s	III	4-piperidinopiperidinyl	0.068	1.592	0.756	0.010	4.150
16t	III	1-pyrazolyl	1.124	15.624	1.370	0.568	11.100
17	IV	H	0.339	15.238	1.310	2.220	20.700
18	III	H	0.236	91.325	5.840	1.100	13.100
19	III	OMe	1.132	8.832	8.500	0.370	12.100
20	V		0.115	5.042	2.340	0.478	10.500
23	IV	morpholinyl	0.107	0.458	0.128	0.003	0.470
25a	II	NMe_2	0.250	25.710	2.100	1.756	12.140
25h	II	morpholinyl	0.215	4.182	1.079	1.017	2.015
26	VI	H	0.269	21.657	6.610	0.742	12.540
27	VI	(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl	0.194	2.921	0.353	0.013	2.860

[a] Concentration (μM) needed to inhibit the autophosphorylation of the cytoplasmic domain of EGFR by 50%, as determined from the dose−response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 2-fold difference. [b] Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of HER-2 by 50%, as determined from the dose−response curve. Determinations were done in duplicate and repeat values agreed, on average, with a mean 5-fold difference. [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.

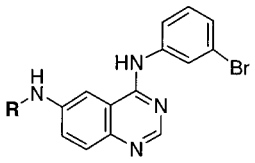
regioisomers **23** and **16h**). The crotonamide **16a** is somewhat more reactive than the butynamide **10a**, while the reverse is true for **1** and **18**. The overall order of reactivity is **23** > **16a** > **10a** > **2** > **16h** for those experiments where no external base had been added.

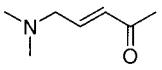
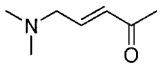
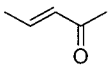
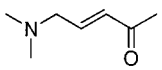
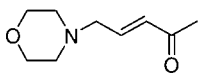
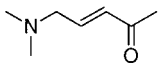
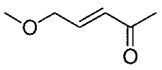
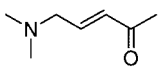
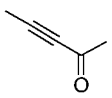
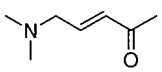
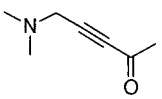
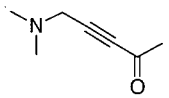
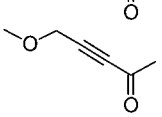
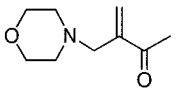
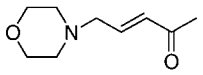
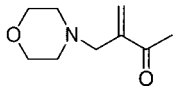
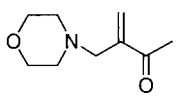
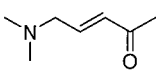
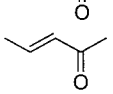
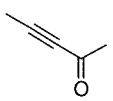
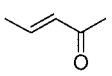
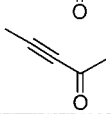
Results and Discussion

Enzyme Inhibitory Activities. The compounds shown in Table 1 were evaluated for their ability to inhibit the autophosphorylation of EGFR and HER-2 kinases using a solid-phase ELISA assay. We note that for compound **2** the IC_{50} for inhibition of EGFR kinase that we measured was 3-fold higher than was found by other researchers.[9] As we pointed out in a previous publication,[20] we attribute this to differences in the nature of the enzyme and substrate as well as to the overall assay conditions, particularly our use of a solid-phase ELISA-based assay. Our enzyme consists of the purified cytoplasmic domain of EGFR and we measure the inhibition of autophosphorylation of this protein. Other researchers have measured inhibition using, in a soluble format, the entire enzyme, purified from A431 cells, along with an exogenous peptide substrate.

As will be shown below for the representative compound **16a**, a number of these inhibitors may bind irreversibly to EGFR (and, presumably, HER-2). This raises some concerns with respect to the meaning of the IC_{50} determinations, since the IC_{50} value should depend on the extent to which the covalent interaction has occurred. In addition, it is conceivable that the IC_{50} value for such an inhibitor will be comprised of two components, one that reflects reversible binding and

Table 2. Competitive Reaction of 6-Substituted-4-(phenylamino)quinazolines with Reduced Glutathione

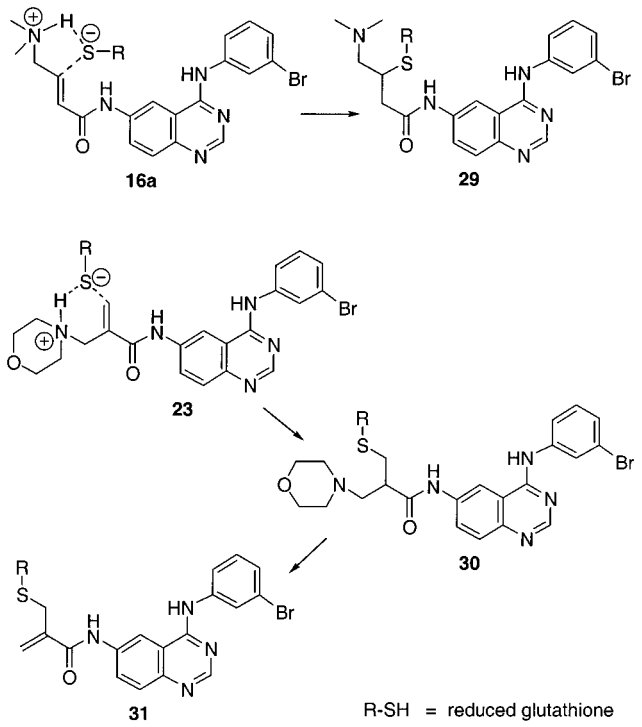


R-				Time[a] (hr)	Glutathione (G) Adduct[b] % conversion	
					A-G	B-G
Compound A		Compound B				
16a		**2**		24	23	2
16a		**18**		24	42	0
16a		**16h**		23	17	0
16a		**19**		17	18	0
16a		**1**		23	31	0
16a		**10a**		24	33	8
10a		**10l**		11	44	0
23		**16h**		24	41	0
23		**17**		24	43	0
23		**16a**		24	39	6
18		**26**		24[c]	8	8
1		**18**		24[c]	42	8
2		**17**		24[c]	44	0
2		**1**		24[d]	36	6

[a] Reaction time at room temperature. [b] Calculated on the basis of the integration of peak areas on the HPLC chromatogram. [c] *N,N*-Diisopropylethylamine (12 equiv) was added. [d] Triethylamine (1000 equiv) was added.

another that reflects the subsequent irreversible covalent binding. One would therefore expect that the IC_{50} values would be time-dependent such that 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}

Scheme 4



value on the reactivity of the inhibitors cannot be counted on due to the component of the IC_{50} that reflects reversible (noncovalent) binding. In addition, one should not expect an ideal correlation between the reactivity of an inhibitor and its IC_{50} value, since the reactivity data alone reveals nothing about the ability of the inhibitor to fit the active site, and if the inhibitor does bind, its reactivity to glutathione in solution does not reveal information about the accessibility of the reactive center to the target sulfhydryl group in the bound state. The situation will be even more complicated in the cellular studies, since in addition to the above factors, it is possible for an inhibitor to be too reactive and be scavenged by nonspecific covalent binding to sulfhydryl-containing species in the cytosol. Nevertheless, we do see an approximate correlation between reactivity and biological activity in this series of compounds.

For a given compound, we observed that the IC_{50} value for inhibition of HER-2 kinase is, in general, higher than that observed for EGFR kinase. This is possibly due, in part, to the fact that in the enzyme assays, we used about a 2.5-fold higher concentration of the purified HER-2 kinase than EGFR kinase. As one might expect in view of the similarity of these two enzymes, there is an approximate correlation between the HER-2 and EGFR inhibitory activities (see Figure 2).

Consider again the pair of compounds **18** and **16a**. The more reactive **16a** is a more potent inhibitor than **18** (by 22-fold in EGFR and 304-fold in HER-2). A similar large difference in activities is observed when comparing **23** with its less reactive counterpart **17**. Conceivably, these large differences in activity stem from **16a** and **23** binding covalently to the enzymes while the less reactive **18** and **17**, if they bind covalently at all, do so much more slowly. With the exception of acrylate **2** and, to some extent, the butynamide **1**, compounds such as **17**, **18**, and **26** that lack a water-solublizing group are among the least active compounds. They are also among the least reactive compounds. Both **1** and **2** have been shown previously to bind covalently to EGFR. The Michael acceptor groups on these two compounds are less sterically constrained compared to **17**, **18**, and **26**, and therefore, this might explain their greater reactivity and biological activity. For the inhibitors which lack a built-in base catalyst, our enzyme model suggests that the basic functional group of Arg 817 is located close enough to the sufhydryl group of Cys 773 to serve as a catalyst for the Michael addition.

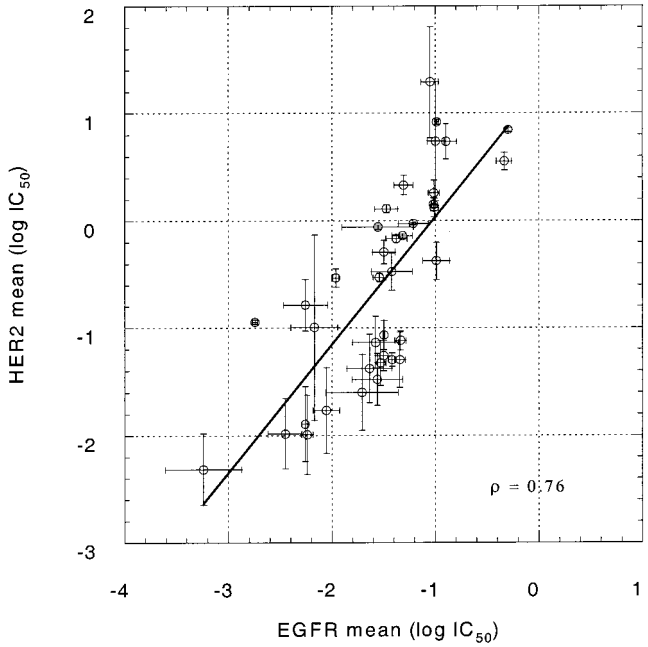


Figure 2. Scatter plot comparing the activities of the compounds in Table 1 against EGFR and HER-2 kinases. The activities are represented as the logarithms of the IC_{50} values measured as micromolar. Each point on the scatter plot is the average of two measurements. Note that error bars are shown for both EGFR and HER-2 activities. The ends of each error bar represent the two log(IC_{50}) measurements on each compound. The least-squares regression line is also shown.

For the butynamide series (series I in Table 1), changing the substituent on the terminal carbon of the Michael acceptor has only a minimal effect on the inhibitory activity for either enzyme, with some indication that larger substituents decrease activity to some extent. Dialkylamino substitution results in a similar situation for the crotonamide series (series III in Table 1), with **16a** having the smaller dimethylamino substituent showing the best activity against EGFR kinase and one of the better activities against HER-2 kinase. While appending hydroxy, methoxy, or polyether groups onto the Michael acceptor in the butynamide series (compounds **10l, 10m, 10n,** and **10o**) is compatible with good activity with both kinases, appending a methoxy group in the crotonamide series as in **19** significantly reduces activities with both enzymes compared to **16a**. This is consistent with the relative reactivities we measured for **16a** and **19**. Moreover, appending a weakly basic pyrazolyl group in the crotonamide series as in **16t** also results in reduced activity compared to **16a**. Attaching a dialkylamino or morpholino moiety directly to the double bond as in **25a** and **25h** (series II in Table 1) results in much less potent compounds when compared to **16a** and **16h**, respectively, where there is

a methylene group between the double bond and the heteroatom. A possible contributing factor for this is the likelihood that the series III compounds are more reactive than the corresponding series II compounds. The configuration of the double bond of the Michael acceptor has little influence on the inhibitory activities with either kinase (compare **18** and **26** or **16j** and **27**) nor does the presence of a chiral center on the water-solublizing moiety (compare **16j** and **16p**). Including the Michael acceptor within a ring, as in **20**, does not result in any significant benefit.

The representative compound **16a** was further evaluated in other kinase assays. Although **16a** is highly potent in inhibiting EGFR and HER-2 kinases, it is much less potent in inhibiting KDR, EcK, Mek/ErK, PDFGR, and VEGF kinases with IC_{50} values greater than 40 μM. This high potency of **16a** in inhibiting EGFR and HER-2 kinases is anticipated from its ability to bind covalently to Cys 773 (Cys 805), which is unique to EGFR (HER-2).

Cell Growth Inhibitory Activity. The compounds were also evaluated for their ability to inhibit the growth of several cell lines, as shown in Table 1. Three human carcinoma cell lines were used: A431 (epidermoid), which overexpresses EGFR; SKBR3 (breast), which overexpresses HER-2 and to a lesser extent, EGFR; and SW620 (colon), which serves as a control line not expressing either EGFR or HER-2 to a significant extent. Several points are apparent from the data shown in Table 1. All our compounds are better inhibitors of the growth of the A431 and SKBR3 cell lines than the SW620 line. This suggests that the mechanism of growth inhibition is due, in large part, to the targeting of EGFR or HER-2 kinases. Second, these compounds inhibit proliferation of SKBR3 cells better than A431 cells. This may reflect the fact that SKBR3 cells, which are slower growing than A431 cells, are more sensitive. Last, the enzyme inhibition data is correlated with cell growth data insofar as the least active enzyme inhibitors, such as **16t**, **17**, **18**, **19**, **25a**, **25h**, and **26**, are among the lesser effective inhibitors of cell growth. We can again compare the two pairs of compounds **16a** and **18** or **23** and **17** and, as expected, based on the reactivity and enzyme data, we find that **16a** and **23** are significantly better inhibitors of the growth of both target cell lines than **18** and **17**, respectively. The presence of water-solublilizing groups on **16a** and **23** could explain part of the enhanced cellular activities by enhancing cellular penetration.

Evidence for Irreversible Binding to EGFR. The correlation we observe between the reactivity of our inhibitors and their ability to inhibit EGFR and HER-2 kinases suggests that the more potent compounds are functioning as irreversible inhibitors. More direct evidence for covalent binding is presented using **16a** as a representative inhibitor.

We compared **16a** with two other compounds, **1**, a known irreversible inhibitor,[10] and **28**, a known reversible inhibitor.[5] These three compounds have comparable IC_{50} values of 0.005, 0.029, and 0.07 μM, respectively, for inhibiting EGFR kinase. As expected, after continuous incubation of A431 cells with drug at a 1 μM concentration for 1 h followed by EGF stimulation, each compound completely inhibited autophosphorylation of EGFR in A431 cells, as shown by Western blot analysis (see Figure 3). However, if the cells were washed continuously for 5 h after drug treatment but prior to EGF stimulation to remove unbound drug, subsequent analysis showed that cells that were treated with **28** had their ability to autophosphorylate EGFR restored, while for cells treated with **16a**, autophosphorylation was completely inhibited just like cells treated with **1**, a known irreversible inhibitor. Other researchers[23] used similar cell-based wash experiments to characterize compounds as irreversible inhibitors when the inhibition of autophosphorylation of EGFR is 80% or greater, with the understanding that this method can generate false positives for irreversible inhibition. In our view, the high reactivity and potency of **16a** in inhibiting EGFR and HER-2 kinases reinforce the observation from the wash experiment. Overall, the results of these experiments with **16a** are consistent with, but do not prove, irreversible inhibition.

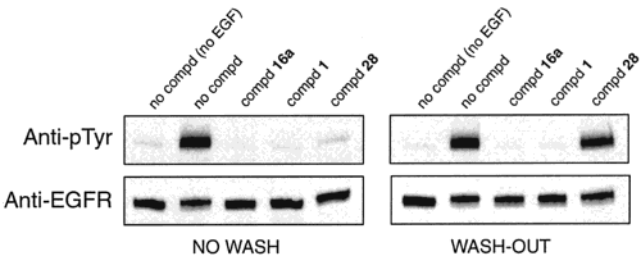


Figure 3. Inhibition of EGFR autophosphorylation in intact A431 cells. Cells were incubated with **1**, **16a**, or **28** at 1 μM concentration and then stimulated with EGF (no wash) or thoroughly washed with fresh medium for 5 h prior to stimulation with EGF (wash-out, top panels). Whole cell lysates were Western blotted using antiphosphotyrosine antibody. Compounds **16a** and **1** both inhibited EGFR autophosphorylation after washout, while **28**, a known reversible compound, showed a full recovery of the ability to autophosphorylate EGFR. Anti-EGFR antibody was used to quantitate EGFR levels (bottom panels).

In Vivo Efficacy. Compounds **16a** and **1** were evaluated in a nude mouse xenograft model bearing A431 human tumors that overexpress EGFR. The results are shown in Figure 4. Drugs were administered orally at 40 mg/kg every day for days 1−10, at which time dosing was discontinued. Both drug-treated groups showed significantly smaller tumors compared to the control group. While compound **1** significantly ($p < 0.01$) inhibited tumor growth by 60−70% by day 10, compound **16a** was capable of inhibiting tumor growth greater than 90%, even 12 days after stopping the drug treatment. Since both compounds **1** and **16a** are expected to be cytostatic agents, once treatment of either drug is stopped, tumor growth should return. However, in comparison with the control group, the tumor growth was still retarded after dosing was discontinued; this is particularly evident with mice treated with **16a**. It is clear that **16a** shows enhanced antitumor activity compared to **1**. It is likely that this enhanced activity is due to the greater reactivity and improved bioavailablity of **16a** compared to **1** (the cLogP value for **1** is 4.45 and the value for **16a** is 3.91).

Conclusions

A series of new quinazoline derivatives that may function as irreversible inhibitors of EGFR and HER-2

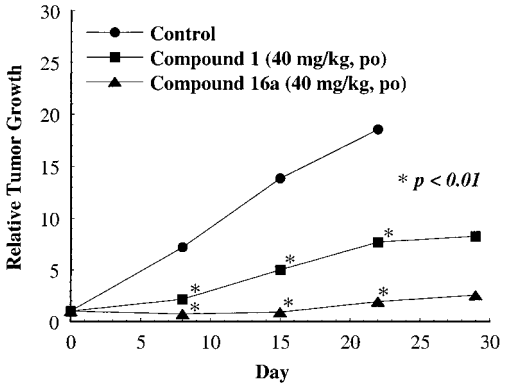


Figure 4. Effects of **1** and **16a** on the growth of the human epidermoid carcinoma A431 in nude mice. Mice were dosed daily with 0.2% Klucel (●), 40 mg/kg **1** (■), or 40 mg/kg **16a** (▲) from day 1 to day 10. Data points represent the mean tumor mass (±SEM) of 10 mice. Relative tumor growth is calculated by mean tumor mass on day 8, 15, 22, or 29 divided by mean tumor mass on day 0. A *p*-value ($p < 0.05$) indicates a statistically significant reduction in relative tumor growth of the treated groups compared with the control group.

kinases have been prepared. These inhibitors have, at the C-6 position, butynamide, crotonamide, and methacrylamide Michael acceptors bearing water-solublilizing substituents. We have shown that attaching a basic functional group onto the Michael acceptor results in greater reactivity due to intramolecular catalysis of the Michael addition and/or increased electrophilicity of the double bond. This, along with improved water-solubility, results in compounds with enhanced biological properties. We have presented molecular modeling and experimental evidence that these inhibitors may interact covalently with the target enzymes. One compound, **16a**, was shown to have excellent oral activity in a human xenograft model of cancer.

Experimental Section

Molecular Modeling. Homology modeling was carried out using the MOE (Molecular Operating Environment) software (Chemical Computing Group Inc., 1010 Sherbrooke Street W., Suite 910, Montreal, Quebec, Canada H3A 2R7). Initial crude minimization was performed within the homology modeling function of MOE. However, the resulting model needed further energy minimization. This was done using Quanta/CHARMm (Molecular Simulations Inc. 9685 Scranton Road, San Diego CA 92121). The crude model from MOE was minimized with a few thousand cycles of minimization using the ABNR (adopted-basis Newton−Raphson) method.

Ligands were modeled by positioning them in the active site in accordance with the published crystal structures of quinazoline derivatives bound to CDK2 and MAP kinase (p38).[24] A 7.5 Å sphere of water molecules was added around the ligand. The entire complex was then subjected to alternate cycles of minimization and dynamics. Each dynamics run was short, about 3ps. The intent was to get a satisfactory structure for the complex that was consistent with the published crystal structures.

Biology. Preparation, and Purification of HER-2 and EGFR DNA Constructs. A 1.7 Kb cDNA encoded for human HER-2 cytoplasmic domain (HER-2-CD, amino acids 676−1245) and 1.6 kb cDNA encoded for the EGFR cytoplasmic domain (EGFR−CD, amino acids 645−1186) were cloned into baculoviral expression vectors pBlueBacHis2B (Invitrogen, Carlsbad, CA) and pFASTBacHTc (GIBCO, Rockville, MD), separately. A sequence that encodes $(His)_6$ was located at the 5′ upstream to the HER-2 and EGFR sequences. Sf-9 cells were infected at moi = 10 for 3 days for protein expression. Sf-9 cell pellets were solubilized at 0 °C in a buffer at pH 7.4 containing 50 mM HEPES, 10 mM NaCl, 1% Triton, 10 μM ammonium molybdate, 100 μM sodium vanadate, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin, and 16 μg/mL benzamidine HCl for 20 min followed by 20 min centrifugation. Crude extract supernatant was passed through an equilibrated Ni−NTA superflow packed column (Qiagen, Valencia, CA) and washed with 10 mM and then 100 mM imidazole to remove nonspecifically bound material. Histidine-tagged proteins were eluted with 250 and 500 mM imidazole and dialyzed against 50 mM NaCl, 20 mM HEPES, 10% glycerol, and 1 μg/mL each of aprotinin, leupeptin, and pepstatin for 2 h. The entire purification procedure was performed at 4 °C or on ice.

Purified HER-2-CD fractions (62.5 kDa) were pooled and 20 μL was subjected to electrophoresis followed by Coomassie blue stain to determine the purity (approximately 80%) of the preparation and Western blot for the identity of protein. The final concentration of the purified HER-2 enzyme was determined to be 0.1 μg/μL by Bradford protein assay. The same procedure was used to determine the EGFR enzyme concentration (62.2 kDa) as 0.04 μg/μL with a purity of approximately 80%. Enzyme preparations were stored at −80 °C.

EGFR and HER-2 Kinase Autophosphorylation Assays by DELFIA/Time-Resolved Fluorometry. Both EGFR and HER-2 kinase assays were set up to assess the level of autophosphorylation based on DELFIA/time-resolved fluorometry. Compounds were dissolved in 100% DMSO and diluted to the appropriate concentrations with 25 mM HEPES at pH 7.4. In each well, 10 μL of compound was incubated with 10 μL (12.5 ng for HER-2 or 5 ng for EGFR) of recombinant enzyme (1:80 dilution in 100 mM HEPES) for 10 min at room temperature. Then, 10 μL of 5× buffer (containing 20 mM HEPES, 2 mM $MnCl_2$, 100 μM Na_3VO_4, and 1 mM DTT) and 20 μL of 0.1 mM ATP-50 mM $MgCl_2$ was added for 1 h. Positive and negative controls were included in each plate by incubation of enzyme with or without ATP−$MgCl_2$. At the end of incubation, liquid was aspirated, and plates were washed three times with wash buffer. A 75 μL (400 ng) sample of europium-labeled anti-phosphotyrosine antibody was added to each well for another 1 h of incubation. After washing, enhancement solution was added and the signal was detected by Victor (Wallac Inc.) with excitation at 340 nm and emission at 615 nm. All reagents were supplied by Wallac Inc. (Wallac/Perkin-Elimer, Gaithersburg, MD). The percentage of autophosphorylation inhibition by the compounds was calculated using the following equation: 100% − [(negative control)/(positive control − negative control)]. The IC_{50} was obtained from curves of percentage inhibition with eight concentrations of compound. As the contaminants in the enzyme preparation are fairly low, the majority of the signal detected by the anti-phosphotyrosine antibody is from EGFR or HER-2. The IC_{50} values reported in Table 1 are averages of duplicate determinations.

Cell Proliferation Assay. Three human carcinoma cell lines, A431 (epidermoid carcinoma), SKBR3 (breast carcinoma), and SW620 (colon carcinoma), were used for the cell proliferation assays. All cell lines were obtained from the American Type Culture Collection. Cells were maintained in RPMI-1640 medium supplemented with 5% fetal bovine serum. Cells were plated in 96-well plates at the densities of 5.0×10^4/mL. On the next day, compounds were dosed at 0.5, 5, 50, 500, and 5000 ng/mL concentrations and the cells were cultured for 2 days. At the end of incubation, cell survival was determined by the sulforhodamine B assay as previously described.[25] The IC_{50} values were obtained from the growth curves.

Competitive Reaction with Reduced Glutathione. Sample solutions were prepared by dissolving two quinazoline compounds (0.0146 mmol for each compound) in 1.75 mL of THF−MeOH (1:2) solution. Glutathione solution was prepared by dissolving 18 mg (0.0586 mmol) of glutathione in 0.5 mL of water. A 300 μL aliquot of sample solution (2.5 μmol for each quinazoline compound) was dispensed into a 2 mL LC analysis vial and diluted with 689 μL of THF−MeOH−H_2O (1:2:1),

followed by addition of 11 μL (1.25 μmol) of freshly prepared glutathione solution. A 5 μL aliquot of the reaction solution was autoinjected into a HP 1100 LC/MS every hour for 24 h. The LC column used was LUNA: ODS3 (4.6 × 150 mm, 5 μm), and the mobile phase was a gradient of 10−40% aqueous formic acid (0.05%) in acetonitrile in 12 min at a flow rate of 1 mL/min. Both a photodiode array detector and a triple quad electrospray mass spectrometer were used for detection.

Inhibition of EGFR Tyrosine Phosphorylation, Immunoprecipitation, and Western Blot Analysis. A431 cells (1×10^6) were seeded in 6-well plates overnight. Cells were exposed to 1 μM compound for 1 h at 37 °C and then either immediately treated with media containing EGF (100 ng/mL) for 15 min or thoroughly washed with fresh medium for 5 h before EGF treatment. Whole cell lysates were prepared and total protein concentrations were determined. The total protein (25 μg) was loaded on 7.5% SDS−PAGE gels for electrophoresis and was subjected to transfer onto PVDF membranes. Membranes were Western blotted first by anti-phosphotyrosine antibody (Transduction Laboratories, Lexington, KY), stripped, and reprobed with anti-EGFR antibody (Transduction Laboratories). The intensity of each band, corresponding to tyrosine phosphorylated EGFR and EGFR protein, was scanned by a Fluor-S MultiImager (BioRad, Hercules, CA). The levels of phosphorylated bands were normalized by the EGFR protein.

In Vivo Evaluation.Compounds **1** and **16a** were evaluated in vivo using a standard pharmacological test procedure that measures the ability to inhibit the growth of human tumor xenografts. The human epidermoid carcinoma A431 (American Type Culture Collection, Rockville, Maryland # CRL-155) was grown in tissue culture in DMEM (Gibco/BRL, Gaithersburg, MD) supplemented with 10% FBS (Gemini Bio-Products Inc., Calabasas, CA). Athymic nu/nu female mice (Charles River, Wilmington, MA) were injected sc in the flank area with 5×10^6 A431 cells. When tumors attained a mass between 80 and 120 mg, the mice were randomized into treatment groups (day zero), 10 animals per group (5 animals per cage). Animals were treated orally once a day on days 1−10 poststaging (day zero) with doses of 40 mg/kg of either compound **1** or compound **16a** prepared in 0.2% Klucel or with 0.2% Klucel as the vehicle control. Tumor mass was determined every 7 days [(length × width^2)/2] starting on day 8 for 29 days poststaging. Relative tumor growth (mean tumor mass on days 8, 15, 22, and 29 divided by the mean tumor mass on day zero) was determined for each treatment group. Statistical analysis (Student-*t*-test) of log(relative tumor growth) was used to compare treated verses control group in each experiment. A *p* value ($p \leq 0.05$) indicated a statistically significant reduction in relative tumor growth of the treated group compared to the vehicle control.

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. HPLC−NMR−MS was performed on a Brucker Avance 600 equipped with a 4 mm ^{1}H/^{13}C flow probe and an in-line HP/Esquire ion trap mass spectrometer operating in positive ion electrospray mode, and the chemical shifts (δ) are referenced against the CH_3CN solvent peak at 1.93 ppm. 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.

***N′*-(2-Cyano-4-nitrophenyl)-*N*,*N*-dimethylimidoformamide (4).** A mixture of 5-nitroanthranilonitrile **3** (192 g, 1.18 mol) and dimethylformamide dimethyl acetal (389 mL) was stirred at reflux for 1.5 h. The resulting mixture was cooled to room temperature and refrigerated. The solid was filtered, washed with several portions of ether, and dried in vacuo (55 °C) to yield 238 g (98%) of product: mp 153−155 °C; ^{1}H NMR (DMSO-d_6) δ 8.48 (d, J = 2.7 Hz, 1H), 8.27 (m, 2H), 7.38 (d, J = 9.3 Hz, 1H), 3.17 (s, 3H), 3.06 (s, 3H). Anal. ($C_{10}H_{10}N_4O_2$) C, H, N.

6-Nitro-4-(3-bromophenylamino)quinazoline (5). A mixture of **4** (228 g, 1.1 mol) and 3-bromoaniline (132 mL, 1.21 mol) in HOAc (1 L) was stirred at reflux for 1 h. The resulting solid was filtered hot and washed with ether. It was dried (50 °C) in vacuo to give 343 g (89%) of the product as a yellow solid: mp 267−270 °C; ^{1}H NMR (DMSO-d_6) δ 10.49 (s, 1H), 9.67 (d, J = 2.1 Hz, 1H), 8.79 (s, 1H), 8.58 (dd, J = 2.4, 9.2 Hz, 1H), 8.20 (s, 1H), 7.98 (s, 1H), 7.93 (dd, J = 2.2, 6.9 Hz, 1H), 7.39 (m, 2H); MS (ESI) m/z 345 $(M + H)^+$. Anal. ($C_{14}H_9BrN_4O_2$) C, H, N.

***N*^{4}-(3-Bromophenyl)-4,6-quinazolinediamine (6).** To a stirred mixture of **5** (150 g, 0.435 mol) in EtOH (750 mL) and HOAc (187 mL) was added Fe (118.8 g, 2.127 mol) and the reaction was heated to reflux. When reflux started, more EtOH (200 mL) and HOAc (46 mL) were added, and reflux was continued for 3 h. The reaction was cooled to room temperature and diluted with water (1 L). The solid was filtered and washed with water until it looked bright yellow. It was then treated with EtOH (75 mL), THF (375 mL), and EtOAc (1050 mL). The insoluble material was filtered off. The filtrate was washed with 25% $KHCO_3$ and twice with brine. It was dried (Na_2SO_4), decolorized with charcoal, and then filtered through Celite. The filtrate was concentrated in vacuo to give 122 g of a yellow solid (89%): mp 203.5−204.5 °C; ^{1}H NMR (DMSO-d_6) δ 9.46 (s, 1H), 8.39 (s. 1H), 8.25 (t, J = 1.8 Hz, 1H), 7.89 (m, 1H), 7.55 (d, J = 8.9 Hz, 1H), 7.29 (m, 4H), 5.64 (s, 2H); MS (ESI) m/z 315 $(M + H)^+$. Anal. ($C_{14}H_{11}BrN_4$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-2-butynamide (1).** To a cold (0 °C) and well-stirred solution of 2-butynoic acid (24.0 g, 0.286 mol) in THF (280 mL) was added dropwise isobutyl chloroformate (37.0 mL, 39.0 g, 0.286 mol) under N_2. This addition was carried out over a period of about 5 min, and the temperature rose to 3 °C. To the above solution was added dropwise *N*-methylmorpholine (31.4 mL, 28.9 g, 0.286 mol) at such a rate that the temperature remained below 10 °C (ca 15 min). After stirring an additional 10 min this mixture was filtered and the cake was washed with THF (40 mL). This combined filtrate was immediately transferred to a dropping funnel on a 2-L three-necked flask that had been previously purged with N_2. This flask contained **6** (60.0 g, 0.19 mol) in pyridine (280 mL) that had been previously cooled to 0−5 °C. The anhydride solution was added dropwise with good stirring at a rate to maintain the temperature at 0−5 °C (ca. 15 min). After stirring for 1 h, the solid was filtered. The product was washed twice with 200 mL portions of water, sucked as dry as possible using a rubber dam, and then washed twice with 200 mL portions of acetonitrile. The wet cake was dried overnight in vacuo (40 °C) to yield 65 g (89%) of pale yellow solid giving a single spot on TLC (1:19 MeOH−CH_2Cl_2, silica gel). The material was 99% pure by analytical HPLC: mp 274−276 °C; ^{1}H NMR (DMSO-d_6) δ 10.96 (s, 1H), 9.93 (s, 1H), 8.76 (s, 1H), 8.59 (s, 1H), 8.15 (d, J = 1.7 Hz, 1H), 7.81 (m, 3H), 7.53 (m, 2H), 2.09 (s, 3H); MS (ESI) m/z 381.383 $(M + H)^+$; HRMS (ESI) m/z calcd for $C_{18}H_{13}BrN_4O$ 381.034 55, found 381.033 63 $(M + H)^+$. Anal. ($C_{18}H_{13}BrN_4O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-acrylamide (2).** A solution of **6** (2.0 g, 6.35 mmol) in pyridine (10 mL) was cooled in an ice bath. To this was added dropwise a solution of acryloyl chloride (0.61 mL, 0.68 g, 7.5 mmol) in ether (30 mL). After stirring at room temperature for 3.5 h, the solvents were removed in vacuo. The residue was purified by chromatography (1:33 to 1:9 MeOH−CH_2Cl_2, silica gel) to give 0.2 g (8.5%) of **2**: mp 279 °C; ^{1}H NMR (DMSO-d_6) 10.53 (s, 1 H), 10.32 (s, 1 H), 8.83 (s, 1 H), 8.59 (s, 1 H), 8.18 (s, 1 H), 7.86 (m, 3 H), 7.33 (m, 2 H), 6.54 (dd, 1 H, J = 17.1 Hz, J = 9.9 Hz), 6.35 (dd, 1 H, J = 1.8 Hz, J = 16.8 Hz), 5.85 (dd, 1 H, J = 1.8 Hz, J = 9.9 Hz); MS (ESI) 368.9, 370.9 $(M + H)^+$; HRMS (ESI) m/z calcd for $C_{17}H_{13}BrN_4O$ 369.034 55, found 369.033 73 $(M + H)^+$. Anal. ($C_{17}H_{13}BrN_4O$) C, H, N.

4-(Dimethylamino)-2-butynoic Acid (9a). *n*-BuLi in hexane (2.5 M, 96 mL) was slowly added to 3-(dimethylamino)-1-propyne (20 g, 240 mmol) in THF (100 mL) under N_2. The mixture was stirred for 1 h at −78 °C, then dry CO_2 was passed through the reaction overnight. The resulting solution was poured into water and washed with EtOAc. The aqueous layer was evaporated in vacuo to give the crude acid. This was dissolved in MeOH, and the insoluble salts were removed via filtration. The filtrate was evaporated to give 15.6 g of **9a**: ^{1}H NMR (DMSO-d_6) δ 4.4 (b, 1H), 3.29 (s, 2H), 2.21 (s, 6H); MS (ESI) *m*/*z* 125.6 $(M - H)^-$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(dimethylamino)-2-butynamide (10a).** Compound **9a** (40.4 mg, 0.318 mmol) was stirred in THF (6 mL). To this was added *N*-methylmorpholine (64.4 mg, 0.636 mmol) and the mixture was cooled to −10 °C. Isobutylchloroformate (34.7 mg, 0.254 mmol) was added and the mixture was stirred for 0.5 h. Compound **6** (50 mg, 0.159 mmol) was dissolved in pyridine (2 mL) and added dropwise to the reaction mixture, which was stirred for 1 h after addition. The reaction was quenched with ice water and saturated $NaHCO_3$ solution and extracted with EtOAc. The organic layer was dried and evaporated to give the crude product, which was purified by preparative thin-layer chromatography (15% MeOH in EtOAc), to give 27 mg (37%) of a pale yellow solid: mp 203−206 °C; ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H), 9.92 (s, 1H), 8.73 (s, 1H), 8.59 (s, 1H), 8.15 (s, 1H), 7.87−7.78 (m, 3H), 7.37−7.28 (m, 2H), 3.50 (s, 2H), 2.27 (s, 6H); MS (ESI) *m*/*z* 424.0 $(M + H)^+$; HRMS (ESI) *m*/*z* calcd for $C_{20}H_{18}BrN_5O$ 424.076 75, found 424.076 86 $(M + H)^+$. Anal. ($C_{20}H_{18}BrN_5O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(diethylamino)-2-butynamide (10b).** By the procedure described above for **10a** using **6** (1.5 g, 4.76 mmol) and **9b** (2.2 g, 14.3 mmol, prepared from 3-(diethylamino)-1-propyne in a manner similar to **9a**), 750 mg (35%) of the title compound was obtained as an olive green solid: mp 114−116 °C; ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 9.92 (s, 1H), 8.71 (s, 1H), 8.59 (s, 1H), 8.16 (s, 1H), 7.88−7.78 (m, 3H), 7.38−7.28 (m, 2H), 3.63 (s, 2H), 2.54 (q, *J* = 7.1 Hz, 4H), 1.03 (t, *J* = 7.1 Hz, 6H); MS (ESI) *m*/*z* 452.4 $(M + H)^+$; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{22}BrN_5O$ 452.108 05, found 452.108 16 $(M + H)^+$. Anal. ($C_{22}H_{22}BrN_5O \cdot 0.75H_2O$) C, H, N.

4-(Dipropylamino)-2-butynoic Acid (9c). To a mixture of Cs_2CO_3 (46.9 g, 0.144 mol), acetone (300 mL), and dipropylamine (19.75 mL, 0.144 mol) was dropwise added propargyl bromide **7** (16.0 mL, 0.144 mol). After the mixture was stirred overnight at room temperature, it was filtered and the acetone solution was evaporated to dryness. The residue was partitioned between EtOAc and aqueous $NaHCO_3$ solution. The organic layer was dried and evaporated to give 12.37 g (62%) 3-(dipropylamino)-1-butyne **8c** as an orange-brown oil: ^{1}H NMR (DMSO-d_6) δ 3.32 (d, *J* = 2.34 Hz, 2H), 3.03 (t, *J* = 2.34 Hz, 1H), 2.36 (t, *J* = 7.08 Hz, 4H), 1.38 (m, 4H), 0.837 (t, *J* = 7.38 Hz, 6H); MS (ESI) *m*/*z* 139.9 $(M + H)^+$.

Compound **8c** was carboxylated in the same manner as **9a** to give **9c** as a tan solid: ^{1}H NMR (DMSO-d_6) δ 3.40 (s, 2H), 2.37 (t, *J* = 7.47 Hz, 4H), 1.38 (m, 4H), 0.841 (t, *J* = 7.35 Hz, 6H); MS (ESI) *m*/*z* 184.0 $(M + H)^+$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(dipropylamino)-2-butynamide (10c).** The title compound was prepared by the reaction of **6** and **9c** using the mixed anhydride procedure as previously described for **10a**. The chromatography solvent was 3% MeOH in EtOAc and a 51% yield of brown crystals was obtained: ^{1}H NMR (DMSO-d_6) δ 10.80 (s, 1H), 9.91 (s, 1H), 8.72 (s, 1H), 8.60 (s, 1H), 8.16 (s, 1H), 7.82 (m, 3H), 7.33 (m, 2H), 3.62 (s, 2H), 2.48 (t, *J* = 7.17 Hz, 4H), 1.45 (m, 4H), 0.88 (t, *J* = 7.29 Hz, 6H); HRMS (CI) *m*/*z* calcd for $C_{24}H_{27}BrN_5O$ 480.1399, found 480.1376 $(M + H)^+$. Anal. ($C_{24}H_{26}BrN_5O \cdot 0.3H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[isopropyl(methyl)amino]-2-butynamide (10d).** By the procedure described above for **10a** using **6** (1.5 g, 4.76 mmol) and 4-[isopropyl(methyl)amino]-2-butynoic acid **9d** (1.5 g, 9.67 mmol, prepared from 3-[isopropyl(methyl)amino]-1-propyne in a manner similar to **9c**), 870 mg (40%) of the title compound was obtained as a pale brown solid: mp 159−160 °C; ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H), 9.80 (s, 1H), 8.60 (s, 1H), 8.47 (s, 1H), 8.04 (s, 1H), 7.75−7.66 (m, 3H), 7.25−7.16 (m, 2H), 3.45 (s, 2H), 2.69 (m, 1H), 2.17 (s, 3H), 0.90 (d, *J* = 6.6 Hz, 6H); MS (ESI) *m*/*z* 452.1 $(M + H)^+$; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{22}BrN_5O$ 452.10805, found 452.108 08 $(M + H)^+$. Anal. ($C_{22}H_{22}BrN_5O \cdot 0.25H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(diisopropylamino)-2-butynamide (10e).** By the procedure described above for **10a**, 1.64 g (72%) of a brown solid was obtained from **6** (1.5 g, 4.76 mmol) and 4-(diisopropylamino)-1-butynoic acid **9e** (1.8 g, 9.60 mmol, prepared in the same manner as **9c**): mp 100 °C (dec); ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H), 9.91 (s, 1H), 8.72 (s, 1H), 8.59 (s, 1H), 8.15 (s, 1H), 7.86−7.77 (m, 3H), 7.37−7.28 (m, 2H), 3.61 (s, 2H), 3.16 (m, 2H), 1.07 (t, *J* = 6.3 Hz, 12H); MS (ESI) *m*/*z* 480.1 $(M + H)^+$; HRMS (ESI) *m*/*z* calcd for $C_{24}H_{26}BrN_5O$ 480.139 35, found 480.139 26 $(M + H)^+$. Anal. ($C_{24}H_{26}BrN_5O \cdot 0.5H_2O$) C, H, N.

4-[Allyl(methyl)amino]-*N*-{4-[(3-bromophenyl)amino]-6-quinazolinyl}-2-butynamide (10f). Using the procedure described for the preparation of **10a**, 750 mg (35%) of a red-brown solid was obtained from **6** (1.5 g, 4.76 mmol) and 4-[allyl-(methyl)amino]-2-butynoic acid **9f** (1.53 g, 10.0 mmol, prepared from allyl(methyl)amine in a manner similar to **9c**): mp 167−169 °C; ^{1}H NMR (DMSO-d_6) δ 11.20 (s, 1H), 10.07 (s, 1H), 8.87 (s, 1H), 8.73 (s, 1H), 8.30 (s, 1H), 8.00−7.92 (m, 3H), 7.52−7.42 (m, 2H), 6.01−5.92 (m, 1H), 5.42−5.30 (m, 2H), 3.70 (s, 2H), 3.23 (d, *J* = 6.0 Hz, 2H), 2.42 (s, 3H); MS (ESI) 450.4 $(M + H)^+$; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{20}BrN_5O$ 450.092 40, found 450.092 30 $(M + H)^+$. Anal. ($C_{22}H_{20}BrN_5O \cdot 0.33H_2O$) C, H, N.

4-(1-Piperidinyl)-2-butynoic Acid (9g). By the procedure described above for **8c**, 12.5 g (63%) of 3-(1-piperidinyl)-1-propyne **8g** was obtained as a brown liquid from piperidine (13.8 g, 162 mmol), propargyl bromide **7** (19.3 g, 162 mmol), and Cs_2CO_3 (52.8 g, 162 mmol): ^{1}H NMR (DMSO-d_6) δ 3.20 (d, *J* = 2.4 Hz, 2H), 3.09 (t, *J* = 2.4 Hz, 1H), 2.36 (m, 4H), 1.49 (m, 4H), 1.33 (m, 2H); MS (ESI) *m*/*z* 123.8 $(M + H)^+$.

Using the procedure described above for **9c**, **8g** (12.2 g, 99.0 mmol) was then converted to 16.5 g (100% crude yield) the title compound **9g** as a light tan foam: ^{1}H NMR (DMSO-d_6) δ 4.49 (bs, 1H), 3.59 (s, 2H), 2.64 (m, 4H), 1.56 (m, 4H), 1.42 (m, 2H); MS (ESI) *m*/*z* 166.0 $(M - H)^-$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(1-piperidinyl)-2-butynamide (10g).** By the procedure described above for **10a** using **6** (1.00 g, 3.17 mmol) and **9g** (1.32 g, 7.92 mmol), the crude product was obtained and chromatographed (15% MeOH in EtOAc) to give 1.07 g (73%) of light green crystals: ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 9.92 (s, 1H), 8.73 (s, 1H), 8.60 (s, 1H), 8.16 (s, 1H), 7.82 (m, 3H), 7.33 (m, 2H), 3.51 (s, 2H), 3.34 (s, 4H), 1.56 (m, 4H), 1.41 (m, 2H); HRMS (EI) *m*/*z* calcd for $C_{23}H_{22}BrN_5O$ 463.1008, found 463.1009 $(M^{+\bullet})$. Anal. ($C_{23}H_{22}BrN_5O \cdot 0.5H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(4-morpholinyl)-2-butynamide (10h).** Using the procedure described for the preparation of **10a**, 90 mg (50%) of the product was obtained from **6** (100 mg, 0.317 mmol) and 4-(4-morpholinyl)-2-butynoic acid **9h (**180 mg, 0.952 mmol, prepared in a manner similar to **9c**): mp 249−252 °C; ^{1}H NMR (DMSO-d_6) δ 10.81 (s, 1H), 9.69 (s, 1H), 8.48 (s, 1H), 8.35 (s, 1H), 7.91 (s, 1H), 7.64−7.54 (m, 3H), 7.13−7.03 (m, 2H), 3.62 (d, *J* = 4.4 Hz, 4H), 3.56 (s, 2H), 2.54 (t, *J* = 4.4 Hz, 4H); MS (ESI) *m*/*z* 466.1 $(M + H)^+$. Anal. ($C_{22}H_{20}BrN_5O_2 \cdot H_2O$) C, H; N: calcd, 14.46; found, 13.96.

4-(4-Thiomorpholinyl)-2-butynoic Acid (9i). By the procedure described above for **8c**, 24.2 g (95%) of 3-(4-thiomorpholinyl)-1-propyne **8i** was obtained as a brown liquid from thiomorpholine (18.2 g, 177 mmol), propargyl bromide **7** (21.0 g, 176 mmol), and Cs_2CO_3 (57.7 g, 177 mmol): ^{1}H NMR (DMSO-d_6) δ 3.27 (d, *J* = 2.4 Hz, 2H), 3.16 (t, *J* = 2.4 Hz, 1H), 2.64 (m, 8H); MS (ESI) *m*/*z* 142.0 $(M + H)^+$.

Using the procedure described above for **9c**, **8i** (23.4 g, 166 mmol) was then converted to the title compound **9i** (30.7 g,

100% crude yield) as an off-white foam: ^{1}H NMR (DMSO-d_6) δ 3.24 (s, 2H), 2.63 (m, 8H); MS (ESI) *m/z* 185.8 $(M + H)^+$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(4-thiomorpholinyl)-2-butynamide (10i).** This compound was prepared by the reaction of **6** (1.00 g, 3.17 mmol) and **9i** (1.47 g, 7.92 mmol) using the mixed anhydride procedure as previously described for **10a**. The chromatography solvent was 5−10% MeOH in EtOAc and 0.513 g (34%) of off-white crystals was obtained: ^{1}H NMR (DMSO-d_6) δ 11.20 (s, 1H), 9.92 (s, 1H), 8.72 (s, 1H), 8.60 (s, 1H), 8.16 (s, 1H), 7.82 (m, 3H), 7.33 (m, 2H), 3.59 (s, 2H), 2.78 (m, 4H), 2.67 (m, 4H); HRMS (EI) *m/z* calcd for $C_{22}H_{20}BrN_5OS$ 481.0573, found 481.0581 ($M^{+\bullet}$). Anal. ($C_{22}H_{20}BrN_5OS\cdot0.5H_2O$) C, H, N.

4-[(2*S*)-2-(Methoxymethyl)-1-pyrrolidinyl]-2-butynoic Acid (9j). By the procedure described above for **8c**, 3-[(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl]-1-propyne **8j** (5.8 g, 90% crude yield) was prepared as a yellow-orange oil from propargyl bromide **7** (5.00 g, 41.9 mmol), (2*S*)-2-(methoxymethyl)-1-pyrrolidine (4.82 g, 41.9 mmol), and Cs_2CO_3 (13.7 g, 41.9 mmol): ^{1}H NMR (DMSO-d_6) δ 3.47 (m, 2H), 3.32 (m, 1H), 3.23 (s, 3H), 3.17 (m, 1H), 3.06 (m, 1H), 2.87 (m, 1H), 2.74 (m, 1H), 2.46 (m, 1H), 1.82 (m, 1H), 1.66 (m, 2H), 1.47 (m, 1H); MS (ESI) *m/z* 153.8 $(M + H)^+$. $[\alpha]_D^{25} = -76$ (*c* 1.29, MeOH).

Using the procedure described above for **9c**, compound **8j** (5.49 g, 35.9 mmol) was then converted to **9j** (7.06 g, 99% crude yield) as a brown foam: ^{1}H NMR (DMSO-d_6) δ 4.36 (bs, 1H), 3.52 (m, 2H), 3.33 (m, 1H), 3.24 (s, 3H), 3.18 (m, 1H), 2.80 (m, 2H), 2.51 (m, 1H), 1.84 (m, 1H), 1.65 (m, 2H), 1.45 (m, 1H); MS (ESI) *m/z* 198.0 $(M + H)^+$. $[\alpha]_D^{25} = -63$ (*c* 0.576, MeOH).

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl]-2-butynamide (10j).** This compound was prepared by the reaction of **6** (1.00 g, 3.17 mmol) and **9j** (1.56 g, 7.94 mmol) using the mixed anhydride procedure as previously described for **10a**. The chromatography solvent was 5−10% MeOH in EtOAc, and 0.570 g (36%) of brown foam was obtained: ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 9.92 (s, 1H), 8.73 (s, 1H), 8.60 (s, 1H), 8.16 (s, 1H), 7.82 (m, 3H), 7.31 (m, 2H), 3.77 (m, 2H), 3.29 (m, 5H), 2.98 (m, 1H), 2.84 (m, 1H), 2.55 (m, 1H), 1.91 (m, 1H), 1.72 (m, 2H), 1.53 (m, 1H); HRMS (EI) *m/z* calcd for $C_{24}H_{24}BrN_5O_2$ 493.1113, found 493.1151 ($M^{+\bullet}$). $[\alpha]_D^{25} = -40$ (*c* 0.761, MeOH). Anal. ($C_{24}H_{24}BrN_5O_2\cdot0.7H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(4-ethyl-1-piperazinyl)-2-butynamide (10k).** By the procedure described above for **10a**, 50 mg (64%) of a bright yellow solid was obtained from **6** (50 mg, 0.159 mmol) and 4-(4-ethyl-1-piperazinyl)-2-butynoic acid **9k** (62 mg, 0.317 mmol, prepared in a manner similar to **9c**): mp 152 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.10 (s, 1H), 9.96 (s, 1H), 8.76 (s, 1H), 8.59 (s, 1H), 8.18 (s, 1H), 7.91−7.78 (m, 3H), 7.38−7.28 (m, 2H), 3.55 (s, 2H), 2.56−2.41 (m, 8H), 2.32 (q, J = 7.0 Hz, 2H), 0.99 (t, J = 7.0 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{24}H_{25}BrN_6O$ 493.134 60, found 493.134 57 $(M + H)^+$. Anal. ($C_{24}H_{25}BrN_6O\cdot1.1H_2O$) C, H; N: calcd, 16.38; found, 15.91.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-methoxy-2-butynamide (10l).** Using the procedure described above for the preparation of **10a**, 68 mg (52%) of a pale yellow solid was obtained from **6** (100 mg, 0.32 mmol) and 4-methoxy-2-butynoic acid **9l** (73 mg, 0.64 mmol, prepared from 3-methoxy-1-propyne **8l** in a manner similar to **9a**): mp 230 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.05 (s, 1H), 9.94 (s, 1H), 8.87 (s, 1H), 8.59 (s, 1H), 8.15 (s, 1H), 7.86−7.78 (m, 3H), 7.40−7.24 (m, 2H), 4.38 (s, 2H), 3.33 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{19}H_{15}BrN_4O_2$ 411.045 12, found 411.044 54 $(M + H)^+$. Anal. ($C_{19}H_{15}BrN_4O_2\cdot0.75H_2O$) C, H, N.

4-(2-Methoxyethoxy)-2-butynoic Acid (9m). To a suspension of 60% NaH in mineral oil (6 g, 151 mmol) in THF (200 mL) at 0 °C with stirring under N_2 was added dropwise methoxyethanol (10 g, 131.4 mmol) over 15 min. The mixture was stirred an additional 1 h. To the stirred mixture at 0 °C was added propargyl bromide **7** (80% in toluene; 14.6 mL, 131.4 mmol). Stirring was continued at room-temperature overnight. The mixture was filtered and the solvent was evaporated. The residue was distilled to give 3-(2-methoxyethoxy)-1-propyne **8m** (11.4 g, 99.9 mmol), which was dissolved in ether (250 mL). The solution was stirred under N_2 and cooled to −78 °C as *n*-BuLi (2.5 M in hexanes, 40 mL, 99.9 mmol) was added over 15 min. Stirring was continued for another 1.5 h. Dry CO_2 was passed over the surface of the stirred reaction as it warmed from −78 °C to room temperature. The mixture was stirred under a CO_2 atmosphere overnight. The mixture was poured into 3 N HCl (100 mL) and solid NaCl was added. The organic layer was separated and dried ($MgSO_4$). The solvent was evaporated and the residue was maintained at 100 °C and 4 mm for 1 h, giving 11.4 g of **9m,** which solidified on storage: ^{1}H NMR ($CDCl_3$) δ 6.00 (bs, 1H), 4.43 (s, 2H), 3.65 (AA′BB′, 4H), 3.42 (s, 3H); MS (ESI) *m/z* 156.8 $(M - H)^-$, 315.0 $(2M - H)^-$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(2-methoxyethoxy)-2-butynamide (10m).** To a stirred solution of **9m** (0.72 g, 4.6 mmol) and isobutyl chloroformate (0.57 mL, 4.4 mmol) in THF (15 mL) at 0 °C was added *N*-methylmorpholine (0.5 mL, 4.6 mmol) followed by **6** (1.2 g, 3.81 mmol). Stirring was continued for 1 h at 0 °C and 30 min at room temperature. The mixture was stored overnight at −10 °C. The mixture was poured into saturated $NaHCO_3$ and extracted with EtOAc. The organic solution was dried ($MgSO_4$). The solvent was removed and the residue was chromatographed on silica gel eluting with EtOAc, chloroform, and MeOH solvent mixtures to give 0.55 g of product as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 11.13 (bs, 1H), 9.93 (bs, 1H), 8.75 (bs, 1H), 8.60 (s, 1H), 8.15 (d, J = 1.7 Hz, 1H), 7.81 (m, 3H), 7.33 (m, 2H), 4.45(s, 2H), 3.52 (AA′BB′, 4H), 3.33 (s, 3H); MS (ESI) *m/z* 454.9, 456.9 $(M + H)^+$. Anal. ($C_{21}H_{19}BrN_4O_3\cdot0.4H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(methoxymethoxy)-2-butynamide (10n).** As described above for **10m**, 0.35 g of a tan solid product was obtained from **6** (1.2 g, 3.81 mmol) and 4-(methoxymethoxy)-2-butynoic acid **9n** (0.66 g, 4.57 mmol; prepared in the same manner as **9m**): ^{1}H NMR (DMSO-d_6) δ 11.15 (bs, 1H), 9.94 (bs, 1H), 8.75 (bs, 1H), 8.60 (s, 1H), 8.15 (bs, 1H), 7.82 (m, 3H), 7.32 (m, 2H), 4.72 (s, 2H), 4.47 (s, 2H), 3.33 (s, 3H); MS (ESI) *m/z* 441.0 $(M + H)^+$. Anal. ($C_{20}H_{17}BrN_4O_3$) H, N, Br; C: calcd, 54.44; found, 53.97.

***tert*-Butyl(dimethyl)(2-propynyloxy)silane (12).** To an ice cold solution of *tert*-butyl(dimethyl)silyl chloride (31.8 g, 0.211 mol), triethylamine (23.5 g, 0.23 mol), and 4-(dimethylamino)pyridine (0.103 g, 0.83 mmol) in CH_2Cl_2 (65 mL) was added dropwise propargyl alcohol (10.6 g, 0.192 mol) in CH_2Cl_2 (15 mL) according to a literature procedure.[26] After stirring at room temperature for 21 h, the reaction solution was washed with brine and dried over Na_2SO_4. After distillation, 22.87 g (64%) of **12** was obtained; ^{1}H NMR ($CDCl_3$) δ 4.31 (d, J = 2.4 Hz, 2H), 2.39 (t, J = 2.4 Hz, 1H), 0.91 (s, 9H), 0.12 (s, 6H); MS (CI) *m/z* 171.2 $(M + H)^+$.

4-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-butynoic Acid (13). A solution of **12** (5 g, 29.4 mmol) in THF (50 mL) was added dropwise into 3 M ethereal MeMgBr (11 mL, 294 mmol) at 0 °C. After stirring at 0 °C for 1.5 h and then at room temperature for 2.5 h, a stream of dry CO_2 was passed through the pale yellow solution for 2 h. The solution was treated with aqueous NH_4Cl (2 g in 9 mL of water) and EtOAc (200 mL). The mixture was titrated with 1% HCl to pH 5.0. The EtOAc layer was then washed with water and dried (Na_2SO_4). After evaporation, 6.28 g of **13** was obtained: ^{1}H NMR ($CDCl_3$) δ 6.14−5.54 (bs, 1H), 4.45 (s, 2H), 0.91 (s, 9H), 0.14 (s, 6H); MS (CI) *m/z* 215.1096 $(M + H)^+$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-butynamide (14).** Isobutyl chloroformate (0.639 g, 4.68 mmol) and *N*-methylmorpholine (0.555 g, 5.48 mmol) were added to an ice-cold solution of **13** (1 g, 4.67 mmol) in THF (32 mL) under N_2. After stirring for 30 min, a solution of **6** (0.979 g, 3.11 mmol) in pyridine (4 mL) was added and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with ice water. The mixture was poured into EtOAc and washed with saturated $NaHCO_3$ and brine. The product was collected and purified by flash column chromatography (60% EtOAc in hexane) to give 0.8 g of product

14: ^{1}H NMR ($CDCl_3$) δ 8.76 (s, 1H), 8.73 (b, 2H), 8.11 (m, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.80 (m, 1H), 7.66 (m, 2H), 7.44 (dd, J = 8.8 Hz, J = 2.1 Hz, 1H), 7.39 (m, 1H), 4.51 (s, 2H), 0.95 (s, 9H), 0.20 (s, 6H); HRMS (CI) m/z 511.1145 $(M + H)^+$.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-hydroxy-2-butynamide (10o).** Compound **14** (300 mg, 0.59 mmol) was stirred for 16 h at room temperature in a solution of HOAc (36 mL), water (12 mL), and THF (12 mL). Then EtOAc was added and the solids were filtered off to give 115 mg of crude product. The filtrates were purified by preparative TLC (EtOAc), to give an additional 20 mg of **10o**: mp 213 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.09 (s, 1H), 9.94 (s, 1H), 8.77 (s, 1H), 8.59 (s, 1H), 8.15 (m, 1H), 7.82 (m, 3H), 7.33 (m, 2H), 5.60 (t, J = 6 Hz, 1H), 4.34 (d, J = 6 Hz, 2H); HRMS (ESI) m/z calcd for $C_{18}H_{13}BrN_4O_2$ 397.02947, found 397.02832 $(M + H)^+$. Anal. ($C_{18}H_{13}BrN_4O_2 \cdot 0.4H_2O$) C, H, N.

(2*E*)-4-Bromo-*N*-{4-[(3-bromophenyl)amino]-6-quinazolinyl}-2-butenamide (15). According to the method of Braun,[15] methyl 4-bromo crotonate (11.7 mL, 17.9 g, 0.1 mol) in EtOH (32 mL) and water (93 mL) was cooled to −11 °C. The reaction was stirred vigorously and finely powdered $Ba(OH)_2$ (15.77 g, 0.05 mol) was added portionwise over a period of 1 h. Cooling and vigorous stirring were continued for about 16 h. The reaction mixture was then extracted with Et_2O (100 mL). The aqueous layer was treated with concentrated H_2SO_4 (2.67 mL, 4.91 g, 0.05 mol) and the mixture was extracted with three 100-mL portions of Et_2O. The combined ethereal extracts were washed with brine (50 mL), dried (Na_2SO_4), and evaporated. The residue was taken up in boiling heptane (ca. 400 mL), which was decanted and evaporated to ca. 50 mL. Cooling gave 3.46 g of 4-bromocrotonic acid.

Oxalyl chloride (2.88 mL, 0.033 mol) was added to 4-bromocrotonic acid (2.47 g, 0.015 mol) suspended in CH_2Cl_2 (25 mL). To this was added 3 drops of DMF. After stirring for about 1.5 h, the solvents were removed in vacuo, and the residual oil was dissolved in THF (20 mL). This solution was cooled in an ice bath and a solution of **6** (4.72 g, 0.015 mol) in THF (50 mL) was added dropwise. This was followed at 0 °C by dropwise addition of *N*,*N*-diisopropylethylamine (2.61 mL, 1.99 g; 0.015 mol) in THF (10 mL). After cooling and stirring for 1 h, EtOAc (80 mL) and water (100 mL) were added. The layers were separated, and the aqueous layer was extracted with 1:1 THF−EtOAc (100 mL). The combined organic layers were washed with 50 mL of brine, dried (Na_2SO_4), and evaporated. The solid was triturated for 1 h with EtOAc (100 mL) to give 5.87 g (84%) of product **15** as an inseparable mixture of the bromide and chloride in a 4:1 ratio: mp 260−280° C; ^{1}H NMR (DMSO-d_6) δ 10.64 (s, 1H), 10.15 (s, 1H broad), 8.86 (d, 1H, J = 1.8 Hz), 8.63 (s, 1H), 8.14 (s, 1 H), 7.92 (dd, 1 H, J = 9 Hz, J = 1.8 Hz), 7.83 (m, 2 H), 7.34 (m, 3 H), 6.97 (m, 1 H), 6.50 (m, 1 H), 4.39 (dd, 1 H, J = 6.7 Hz, J = 0.5 Hz). Anal. ($C_{18}H_{14}Br_2N_4O$) C, H, N; Br: calcd, 34.58; found, 33.58. MS (ESI) m/z 460.8, 416.9 $(M + H)^+$.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(dimethylamino)-2-butenamide (16a). Dimethylamine (2 M in THF, 25 mL) was stirred and cooled in an ice bath. A solution of **15** (1.16 g, 2.5 mmol) in THF (20 mL) was added dropwise, and the reaction was stirred for 2 h. Then EtOAc (45 mL) and saturated $NaHCO_3$ (30 mL) were added. The layers were separated, and the organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residual oil was purified by flash chromatography on silica gel (MeOH−CH_2Cl_2, 1:4) to give 0.381 g (56%) of **16a** as a light yellow solid: mp 209−211 °C; ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H), 9.92 (s, 1H), 8.84 (d, J = 1.8 Hz, 1H), 8.58 (s, 1H), 8.18 (t, J = 1.8 Hz, 1H), 7.83 (m, 3H), 7.32 (m, 3H), 6.83 (m, 1H), 3.12 (d, J = 5.1 Hz, 2H), 2.21 (s, 6H); MS (ESI) m/z 426.0, 428.1 $(M + H)^+$. Anal. ($C_{20}H_{20}BrN_5O \cdot 0.25H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(diethylamino)-2-butenamide (16b). A solution of diethylamine (5.17 mL, 3.66 g, 50 mmol) in THF (20 mL) was cooled in an ice bath. A solution of **15** (1.16 g, 2.5 mmol) in THF (10 mL) and DMF (5 mL) was added dropwise. Stirring was continued for 2 h, and the reaction was poured into saturated $NaHCO_3$. This mixture was extracted with EtOAc, and the combined EtOAc extracts were dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (1:19, MeOH-CH_2Cl_2) to give 0.68 g (59%) of **16b** as a brown solid: mp 196−199 °C; ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 9.92 (s, 1H), 8.21 (s, 1H), 8.58 (d, J = 1.2 Hz, 1H), 8.18 (m, 1H), 7.88 (m, 3H), 7.81 (d, J = 3.0 Hz, 1H), 7.32 (m, 3H), 6.87 (m, 1H), 6.39 (d, J = 15.3 Hz, 1H), 3.32 (d, J = 7.1 Hz, 4H), 1.01 (t, J = 6.9 Hz, 6H); MS (ESI) 228.5 $(M + 2H)^{2+}$. Anal. ($C_{22}H_{24}BrN_5O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(dipropylamino)-2-butenamide (16c). Using a procedure similar to that of **16b**, 1.35 g (56%) of the product was obtained from **15** (2.31 g, 5 mmol) and dipropylamine (6.78 g, 67.0 mmol): ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 9.92 (s, 1H), 8.82 (s, 1H), 8.59 (s, 1H), 8.18 (m, 1H), 7.88 (m, 2H), 7.80 (d, J = 9 Hz, 1H), 7.35 (dd, J = 8 Hz, J = 8 Hz, 1H), 7.29 (m, 1H), 6.87 (td, J = 15.3, 5.7 Hz, 1H), 6.37 (d, J = 15.3 Hz, 1H), 3.24 (d, J = 5.7 Hz, 2H), 2.38 (t, J = 7.2 Hz, 4H), 1.41 (m, 4H), 0.87 (t, J = 7.2 Hz, 6H); HRMS (ESI) m/z calcd for $C_{24}H_{28}BrN_5O$ 482.1556, found 482.1547 $(M + H)^+$. Anal. ($C_{24}H_{28}BrN_5O \cdot 0.5H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(1-piperidinyl)-2-butenamide (16g). Using a procedure similar to that of **16b**, 0.903 g (38%) of the product was obtained from **15** (2.31 g, 5 mmol) and piperidine (4.4 g, 50.5 mmol): mp 227−229 °C; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H), 9.93 (s, 1H), 8.82 (s, 1H), 8.59 (s, 1H), 8.18 (s, 1H), 7.83 (m, 3H), 7.33 (m, 2H), 6.82 (m, 1H), 6.39 (d, J = 15.6 Hz, 1H), 3.37 (s, 6H), 1.57 (s, 3H), 1.43 (s, 3H); MS (ESI) m/z 465.9, 468.0 $(M + H)^+$. Anal. ($C_{23}H_{24}BrN_5O \cdot 1.75H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(4-morpholinyl)-2-butenamide (16h). Using a procedure similar to that of **16b**, 0.903 g (38%) of the product was obtained from **15** (2.31 g, 5 mmol) and morpholine (4.4 g, 5.5 mmol): mp 227−229 °C; ^{1}H NMR (DMSO-d_6) δ 10.43 (s, 1H), 9.93 (s, 1H), 8.81 (d, J = 1.8 Hz, 1H), 8.59 (s, 1H), 8.18 (m, 1H), 7.88 (m, 2H), 7.83 (d, J = 8.7 Hz, 1H), 7.33 (m, 2H), 6.83 (m, 1H), 6.38 (d, J = 15.6 Hz, 1H), 3.62 (t, J = 4.5 Hz, 4H), 3.33 (s, 1H), 3.17 (dd, J = 5.8, 1.1 Hz, 1H), 2.42 (t, J = 4.3 Hz, 4H); MS (ESI) m/z 468.0, 470.9 $(M + H)^+$. Anal. ($C_{22}H_{22}BrN_5O_2 \cdot 1.75H_2O$) C, H, N, Br.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(4-thiomorpholinyl)-2-butenamide (16i). Using a procedure similar to that of **16b**, 1.26 g (52%) of the product was obtained from **15** (2.31 g, 5 mmol) and thiomorpholine (4.87 g, 47.2 mmol): ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 9.92 (s, 1H), 8.82 (s, 1H), 8.59 (s, 1H), 8.18 (m, 1H), 7.89 (m, 2H), 7.80 (d, J = 8.7 Hz, 1H), 7.34 (m, 2H), 6.81 (td, J = 15.3, 5.7 Hz, 1H), 6.36 (d, J = 15.3 Hz, 1H), 3.32 (m, 4H), 3.20 (d, J = 5.7 Hz, 2H), 2.66 (m, 4H); HRMS (ESI) m/z calcd for $C_{22}H_{22}BrN_5OS$ 484.0807, found 484.0805 $(M + H)^+$. Anal. ($C_{22}H_{22}BrN_5OS$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl]-2-butenamide (16j). This compound was prepared by the reaction of **15** (0.550 g, 1.19 mmol) and (2*S*)-2-(methoxymethyl)pyrrolidine (0.162 g, 1.43 mmol) as previously described. The chromatography solvent was 20% MeOH in EtOAc, and 0.260 g (44%) of a brown solid was obtained. An analytical sample was prepared by preparative TLC (silica gel, 15% MeOH in $CHCl_3$): ^{1}H NMR (DMSO-d_6) δ 10.45 (s, 1H), 9.93 (s, 1H), 8.84 (s, 1H), 8.58 (s, 1H), 8.17 (s, 1H), 7.87 (m, 2H), 7.79 (d, J = 7.79 Hz, 1H), 7.33 (m, 2H), 6.88 (m, 1H), 6.36 (d, J = 12 Hz, 1H), 3.70 (m, 1H), 3.21 (m, 7H), 2.67 (m, 1H), 2.21 (m, 1H), 1.86 (m, 1H), 1.68 (m, 2H), 1.50 (m, 1H); HRMS (EI) m/z calcd for $C_{24}H_{26}BrN_5O_2$ 495.1261, found 495.1271 ($M^{+\bullet}$). Anal. ($C_{24}H_{26}BrN_5O_2 \cdot 2.9H_2O$) C, H, N; $[\alpha]_D^{25} = 1$ (c 0.415, MeOH).

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[(2*R*)-2-(methoxymethyl)-1-pyrrolidinyl]-2-butenamide (16p). This compound was prepared by the reaction of **15** (0.550 g, 1.19 mmol) and (2*R*)-2-(methoxymethyl)pyrrolidine (0.162 g, 1.43 mmol) as previously described. The chromatography solvent was 20% MeOH in EtOAc and 0.210 g (36%) of

a brown solid was obtained. An analytical sample was prepared by preparative TLC (silica gel, 35% MeOH in $CHCl_3$): ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H), 9.94 (s, 1H), 8.85 (s, 1H), 8.58 (s, 1H), 8.18 (m, 1H), 7.88 (m, 2H), 7.79 (d, J = 8.7 Hz, 1H), 7.33 (m, 2H), 6.91 (m, 1H), 6.37 (d, J = 15.6 Hz, 1H), 3.71 (m, 1H), 3.26 (s, 3H), 3.15 (m, 3H), 3.02 (m, 1H), 2.66 (m, 1H), 2.21 (m, 1H), 1.88 (m, 1H), 1.71 (m, 2H), 1.52 (m, 1H); HRMS (EI) m/z calcd for $C_{24}H_{26}BrN_5O_2$ 495.1261, found 495.1269 ($M^{+\bullet}$). Anal. ($C_{24}H_{26}BrN_5O_2 \cdot 0.6CHCl_3$) C, H, N; N: calcd, 12.32; found, 11.89; $[\alpha]_D^{25}$ = 23 (c 0.449, MeOH).

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[(2-methoxyethyl)(methyl)amino]-2-butenamide (16q). Using a procedure similar to **16b**, 1.21 g (50%) of the product was obtained from **15** (2.31 g, 5 mmol) and (2-methoxyethyl)-(methyl)amine (4.15 g, 46.6 mmol): ^{1}H NMR (DMSO-d_6) 10.42 (s, 1 H), 9.92 (s, 1 H), 8.84 (s, 1 H), 8.58 (s, 1 H), 8.17 (m, 1 H), 7.88 (m, 2 H), 7.80 (d, J = 9 Hz, 1 H), 7.35 (dd, J = 7.8, 7.8 Hz, 1 H), 7.29 (m, 1 H), 6.84 (td, J = 15.3, 5.7 Hz, 1 H), 6.36 (d, J = 15.3 Hz, 1 H), 3.46 (t, J = 5.9 Hz, 2 H), 3.34 (s, 3 H), 3.22 (d, J = 5.7 Hz, 2 H), 2.55 (t, J = 5.9 Hz, 2 H), 2.23 (s, 3 H); HRMS (ESI) m/z calcd for $C_{22}H_{24}BrN_5O_2$ 470.1192, found 470.1177 (M + H)$^+$. Anal. ($C_{22}H_{24}BrN_5O_2 \cdot 0.5\ H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[(2-hydroxyethyl)(methyl)amino]-2-butenamide (16r). This compound was prepared by the reaction of **15** (0.550 g, 1.19 mmol) and (2-hydroxyethyl)(methyl)amine (0.107 g, 1.43 mmol) as previously described for **16b**. The chromatography solvent was 30−35% MeOH in EtOAc, and 0.130 g (24%) of yellow solid was obtained: ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H), 9.93 (s, 1H), 8.84 (s, 1H), 8.58 (s, 1H), 8.19 (m, 1H), 7.89 (m, 2H), 7.79 (d, J = 9 Hz, 1H), 7.32 (m, 2H), 6.86 (m, 1H), 6.36 (d, J = 15.6 Hz, 1H), 4.44 (t, J = 5.4 Hz, 1H), 3.51 (m, 2H), 3.22 (d, J = 5.01 Hz, 2H), 2.46 (t, J = 6.36 Hz, 2H), 2.23 (s, 3H); MS (ESI) m/z 456.0, 457.9 (M + H)$^+$; HRMS (ESI) m/z calcd for $C_{21}H_{22}BrN_5O_2$ 456.102 97, found 456.103 16 (M + H)$^+$. Anal. ($C_{21}H_{22}BrN_5O_2 \cdot 1.2H_2O$) C, H, N; H: calcd, 5.45; found, 5.02.

(2*E*)-4-(1,4′-Bipiperidin-1′-yl)-*N*-{4-[(3-bromophenyl)-amino]-6-quinazolinyl}-2-butenamide (16s). This compound was prepared by the reaction of **15** (0.700 g, 1.52 mmol) and 4-piperidinopiperidine (0.305 g, 1.82 mmol) as previously described for **16b**. The chromatography solvent was 1:1 EtOAc−MeOH, followed by 1:1 EtOAc−MeOH + 1% Et_3N, and 0.300 g (35%) of brown solid was obtained: ^{1}H NMR (DMSO-d_6) δ 10.54 (s, 1H), 9.97 (s, 1H), 8.86 (s, 1H), 8.58 (s, 1H), 8.21 (m, 1H), 7.90 (m, 2H), 7.78 (d, J = 9 Hz, 1H), 7.28 (m, 2H), 6.80 (m, 1H), 6.32 (d, J = 15.3 Hz, 1H), 3.60 (m, 11H), 3.07 (d, J = 5.43 Hz, 1H), 2.86 (d, J = 11.2 Hz, 1H), 1.91 (t, J = 15.9 Hz, 1H), 1.70 (d, J = 11.2 Hz, 1H), 1.45 (m, 6H); HRMS (EI) m/z calcd for $C_{28}H_{33}BrN_6O$ 548.1899, found 548.1882 ($M^{+\cdot}$) Anal. ($C_{28}H_{33}BrN_6O \cdot 1.5H_2O$) C, N; H: calcd, 6.28; found, 5.86.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-(1*H*-pyrazol-1-yl)-2-butenamide (16t). Using the method described above for **15, 6** (3.00 g, 9.52 mmol), *N*,*N*-diisopropylethylamine (1.41 g, 10.95 mmol), and 4-bromocrotonyl chloride (2.01 g, 10.95 mmol) in THF (36 mL) were condensed. Pyrazole (3.89 g, 57.1 mmol) was added and the reaction was refluxed for 8 h. After work up as before and chromatography on silica gel using $CHCl_3$−EtOAc−MeOH mixtures, 1.3 g (31%) of the product was obtained as a light tan solid: ^{1}H NMR (DMSO-d_6) δ 10.48 (bs, 1H), 9.97 (bs, 1H), 8.82 (bs, 1H), 8.60 (s, 1H), 8.17 (bs, 1H), 7.82 (m, 5H), 7.37 (m, 3H), 7.02 (dt, J = 3, 12 Hz, 1H), 6.02 (d, J = 12 Hz, 1H), 5.07 (m, 2H); MS (ESI) m/z 450.9 (M + H)$^+$. Anal. ($C_{21}H_{17}BrN_6O \cdot 0.5\ H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-2-methylacrylamide (17).** A solution of **6** (1.58 g, 5 mmol) in pyridine (15 mL) was cooled in an ice bath and a solution of methacryloyl chloride (0.59 mL, 0.63 g, 6 mmol) in Et_2O (6 mL) was added dropwise. After stirring and cooling for 2 h, the solvents were removed in vacuo. The residue was washed with water and the resulting solid was taken up in warm *n*-BuOH. Addition of Et_2O to the cooled solution gave 0.44 g (23%) of **17**: mp 240−245 °C: ^{1}H NMR (DMSO-d_6) δ 10.19 (s, 1H), 10.15 (d, J = 6.7 Hz, 1H), 8.80 (d, J = 2.1, 1H), 8.61 (s, 1H), 8.18 (s, 1H), 7.97 (d, J = 2.2 Hz, 1H), 7.94 (d, J = 2.2 Hz, 1H), 7.88 (d, J = 7.5 Hz, 1H), 7.33 (m, 2H), 5.94 (d, J = 0.7 Hz, 1H), 5.62 (d, J = 1.02 Hz, 1H), 2.02 (s, 3H); MS (ESI) m/z 383, 385 (M + H)$^+$. Anal. ($C_{18}H_{15}BrN_4O \cdot 0.25H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-2-butenamide (18). A solution of **6** (1.58 g, 5 mmol) and pyridine (0.5 mL, 0.489 g; 6.2 mmol) in *N*-methylpyrrolidone (15 mL) was stirred and cooled in an ice bath. To this was added dropwise a solution of *trans*-crotonyl chloride (0.53 mL, 0.57 g, 5.5 mmol) in CH_3CN (15 mL). Cooling and stirring were continued for 1 h, and the reaction was poured into saturated $NaHCO_3$ (50 mL). The resulting solid was collected and chromatographed on silica gel. Elution with a gradient of 2:1 EtOAc−hexanes to EtOAc gave 1.32 g (68%) of **18** as a white solid: mp 267−269 °C; ^{1}H NMR (DMSO-d_6) δ 10.58 (s, 1H), 8.92 (s, 1H), 8.68 (s, 2H), 8.09 (m, 1H) 7.83 (m, 2H), 7.39 (m, 3H), 6.91 (m, 1H), 6.27 (m, 1H), 1.92 (d, J = 6.7 Hz, 3H); MS (ESI) m/z 383, 385 (M + H)$^+$. Anal. ($C_{18}H_{15}BrN_4O \cdot 0.25\ H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-methoxy-2-butenamide (19). To a stirred solution of **6** (1 g, 3.17 mmol) and *N*,*N*-diisopropylethylamine (0.62 g, 4.80 mmol) in THF (21 mL) at 0 °C was added 4-methoxycrotonyl chloride[17] (0.62 g, 4.76 mmol). The mixture was stirred at 0 °C for 1.5 h and then at room temperature for 10 min. The mixture was poured into saturated $NaHCO_3$−brine and extracted with EtOAc. The organic solution was dried ($MgSO_4$), filtered through silica gel, and evaporated. The residue was recrystallized from *n*-BuOH to give 1.25 g (95%) of **19** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H), 9.95 (bs, 1H), 8.83 (s, 1H), 8.59 (s, 1H), 8.17 (bs, 1H), 7.86 (m, 3H), 7.33 (m, 2H), 6.91 (dt, J = 3.9, 15.6 Hz, 1H), 6.43 (d, J = 15.6 Hz, 1H), 4.15 (m, 2H), 3.33 (s, 3H); MS (ESI) m/z 413 (M + H)$^+$. Anal. ($C_{19}H_{17}BrN_4O_2 \cdot 0.33H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-1-methyl-1,2,5,6-tetrahydro-3-pyridinecarboxamide (20).** Using the method described above for **19**, **6** (0.75 g, 2.38 mmol), *N*,*N*-diisopropylethylamine (1.54 g, 11.9 mmol), and *N*-methyl-1,2,5,6-tetrahydronicotinyl chloride hydrochloride[18] (0.61 g, 3.1 mmol) gave 0.9 g (87%) of **20** as a light yellow powder: ^{1}H NMR (DMSO-d_6) δ 10.11 (bs, 1H), 9.90 (bs, 1H), 8.78 (d, J = 1.8 Hz, 1H), 8.59 (s, 1H), 8.20 (bs, 1H), 7.94 (dd, J = 1.8, 9.0 Hz, 1H), 7.92 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 9 Hz, 1H), 7.30 (m, 2H), 6.87 (t, J = 1.59, 1H), 3.50−2.90 (m's, 6H), 2.35 (s, 3H); MS (ESI) m/z 438.3, 440.3 (M + H)$^+$. Anal. ($C_{21}H_{20}BrN_5O \cdot 0.5H_2O$) C, H, N.

***N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-2-(4-morpholinylmethyl)acrylamide (23).** A solution of 2-(4-morpholinylmethyl)acrylic acid **21**[19] (2.06 g, 12 mmol) in THF (25 mL) was stirred and cooled in an ice bath. To this was added isobutyl chloroformate (1.56 mL, 1.64 g, 12 mmol) and then *N*-methylmorpholine (1.32 mL, 1.22 g, 12 mmol), giving the mixed anhydride **22** as a precipitate. This mixture was stirred for 2 min and then **6** (3.15 g, 10 mmol) in pyridine (25 mL) was added in one portion. The reaction was stirred and cooled for 0.5 h and then it was poured onto ice and EtOAc. The EtOAc layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel. Elution with a gradient from 1:1 EtOAc−hexane to 1:19 EtOAc−MeOH gave 0.733 g (15%) of **23** as a cream-colored solid: mp 158−166 °C; ^{1}H NMR (DMSO-d_6) δ 11.17 (s, 1H), 9.94 (s, 1H), 8.81 (d, J = 1.8 Hz, 1H), 8.60 (s, 1H) 8.19 (dd, J = 4.2 Hz, J = 1.8 Hz, 1H), 7.87 (m, 3H), 7.31 (m, 2H), 6.15 (d, J = 1.2 Hz, 1H), 5.63 (s, 1H), 3.68 (m, 4H), 3.36 (m, 6H); MS (ESI) m/z 468.1 (M + H)$^+$. Anal. ($C_{22}H_{22}BrN_5O_2 \cdot H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-3-(dimethylamino)-2-propenamide (25a). To a stirred solution of **6** (3.0 g, 9.52 mmol) and *N*,*N*-diisopropylethylamine (1.23 g, 9.52 mmol) in THF (26 mL) at 0 °C was added *trans*-3-chloroacryloyl chloride (1.85 g, 14.9 mmol) in THF (5 mL). After 1 h, dimethylamine (2M in THF, 71.4 mL) was added and the mixture was stirred an additional 1.5 h. The solvent was evaporated and the residue was dissolved in a mixture of EtOAc and THF. The solution was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and passed through a short silica gel

column. The solvent was evaporated and the residue was boiled in EtOH. After cooling, 1.2 g (31%) of the product **25a** was collected as a yellow powder: ^{1}H NMR (DMSO-d_6) δ 9.97 (bs, 1H), 9.82 (bs, 1H), 8.72 (d, J = 1.8 Hz, 1H), 8.53 (s, 1H), 8.18 (m, 1H), 7.85 (m, 2 H), 7.72 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 12.6 Hz, 1H), 7.39 (m, 2H), 4.82 (d, J = 12.6 Hz, 1H), 3.32 (s, 6H); MS (ESI) m/z 412.0, 413.9 $(M + H)^+$. Anal. ($C_{19}H_{18}BrN_5O \cdot 0.5H_2O$) C, H, N.

(2*E*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-3-(4-morpholinyl)-2-propenamide (25h). Using the method described above for **25a**, reaction of **6** (3.0 g, 9.52 mmol), *N*,*N*-diisopropylethylamine (1.85 g, 14.3 mmol), and *trans*-3-chloroacryloyl chloride (2.38 g, 19.0 mmol), followed by treatment with morpholine (12.4 g, 142.8 mmol), gave a crude product. After crystallization from EtOH, 1.56 g (36%) of the product **25h** was obtained as an orange powder: ^{1}H NMR (DMSO-d_6) δ 9.84 (bs, 1H), 9.70 (bs, 1H), 8.70 (d, J = 1.8 Hz, 1H), 8.54 (s, 1H), 8.18 (m, 1H), 7.87 (m, 2H), 7.73 (d, J = 9.0 Hz, 1H), 7.35 (m, 3H), 5.04 (d, J = 12.8, 1H) 3.65 (m, 4H), 3.22 (m, 4H); MS (ESI) m/z 454.0, 456.0 $(M + H)^+$. Anal. ($C_{21}H_{20}BrN_5O_2 \cdot 0.66H_2O$) C, H, N.

(2*Z*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-2-butenamide (26). Compound **1** (50 mg, 0.13 mmol) was dissolved in MeOH (10 mL). To this was added Lindlar catalyst (6 mg) and H_2 was passed over the surface of the stirred mixture. The reaction mixture was stirred for 4 h at room temperature, then filtered through Celite, and evaporated to give 38 mg (76%) of **26** as a pale yellow solid: mp 236−238 °C; ^{1}H NMR (DMSO-d_6) δ 10.35 (s, 1H), 9.92 (s, 1H), 8.81 (d, J = 1.2 Hz, 1H), 8.58 (s, 1H), 8.18 (s, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.87 (dd, J = 9, 1.2 Hz, 1H), 7.79 (d, J = 9 Hz, 1H), 7.35 (dd, J = 7.8, 7.8 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 6.32 (qd, J = 11.4, 7 Hz, 1H), 6.10 (dd, J = 11.4, 1.5 Hz, 1H), 2.19 (dd, J = 7, 1.5 Hz, 3H); HRMS (ESI) m/z calcd for $C_{18}H_{15}BrN_4O$ 383.050 20, found 383.049 43 $(M + H)^+$. Anal. ($C_{18}H_{15}BrN_4O \cdot 0.5H_2O$) C, H; N: calcd, 14.28; found, 13.79.

(2*Z*)-*N*-{4-[(3-Bromophenyl)amino]-6-quinazolinyl}-4-[(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl]-2-butenamide (27). Compound **10j** (100 mg, 0.20 mmol) was dissolved in MeOH (20 mL). To this was added Lindlar catalyst (12 mg), and H_2 was passed over the surface of the stirred mixture. The reaction mixture was stirred for 16 h at room temperature, then filtered through a Celite plug, and evaporated to give 95 mg of crude product. Purification was carried out by preparative TLC (20% MeOH in EtOAc) to give 68 mg (68%) of **27**: mp 144−145 °C; ^{1}H NMR (DMSO-d_6) δ 10.49 (s, 1H), 9.92 (s, 1H), 8.80 (s, 1H), 8.58 (s, 1H), 8.17 (m, 1H), 7.86 (m, 2H), 7.79 (d, J = 6 Hz, 1H), 7.35 (m, 2H), 6.34 (m, 1H), 6.14 (d, J = 9 Hz, 1H), 4.02 (m, 1H), 3.77 (m, 1H), 3.32 (s, 3H), 3.23 (s, 2H), 3.04 (m, 1H), 2.63 (m, 1H), 2.23 (m, 1H), 1.83 (m, 1H), 1.66 (m, 2H), 1.49 (m, 1H); HRMS (EI) m/z calcd for $C_{24}H_{26}BrN_5O_2$ 495.1270, found 495.1280 $(M^{+\bullet})$. Anal. ($C_{24}H_{26}BrN_5O_2 \cdot 0.5H_2O$) C, H, N.

***L*-γ-Glutamyl-*S*-{2-[({4-[(3-bromophenyl)amino]quinazolin-6-yl}amino)carbonyl]prop-2-enyl}-*L*-cysteinylglycine (28).** Compound **23** (11.8 mg, 0.025 mmol) and reduced glutathione (11.2 mg, 0.036 mmol) were dissolved in THF (0.87 mL), MeOH (1.75 mL), and H_2O (0.35 mL) followed by addition of triethylamine (5 μL, 0.036 mmol). After stirring at room temperature for 3 d, the solvents were removed by lyophilization, and the crude product was analyzed by HPLC−NMR−MS. A 25 mL CH_3CN solution containing 25 μg of crude product was injected into a HPLC equipped with a Waters X-terra column (4.6 × 150 mm, 5 μm) and eluted with a linear gradient solvent system (90% A−10% B to 30% A−70% B over 17 min at a flow rate of 1.0 mL/min; solvent A, D_2O−0.02% TFA; solvent B, CH_3CN−0.02% TFA). A main peak at 7.7 min that showed m/z 697.9 $(M + H)^+$ was collected in a loop of a Brucker BPSU-36 LC-interface cartridge and flow-injected into the NMR probe with a solvent composition matching that of the eluted peak. The 1-D double NOESY and 2-D TOCSY spectra identified the product as **28**:^{1}H NMR (D_2O−CH_3CN) δ 8.67 (s, 1H), 8.57 (s, 1H), 8.05 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 7.3 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 5.89 (s, 1H), 5.67 (s, 1H), 4.46 (m, 1H), 3.82 (s, 2H), 3.78 (t, J = 6.5 Hz, 1H), 2.88 (m, 1H), 2.71 (m, 1H), 2.40 (t, J = 6.6 Hz, 2H), 2.02 (m, 2H).

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