The Design and Synthesis of Water-Soluble Analogues of CB30865, a Quinazolin-4-one-Based Antitumor Agent

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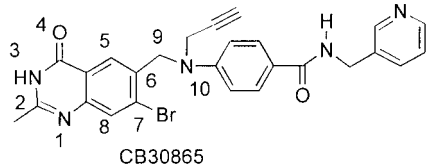
4-[*N*-[7-Bromo-2-methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]-*N*-(3-pyridylmethyl)benzamide (CB30865) is a quinazolin-4-one antitumor agent whose high growth-inhibitory activity (W1L2 $IC_{50} = 2.8 \pm 0.50$ nM) is believed to have a folate-independent locus of action. In addition, CB30865 represents a class of compounds with unique biochemical characteristics such as a delayed, non-phase specific, cell-cycle arrest. The low aqueous solubility of CB30865 prompted a search for more water-soluble analogues for in vivo evaluation of this class of compounds. It was thought that aqueous solubility could be increased by the introduction of amino functionalities at the 2-position of the quinazolin-4-one ring. A variety of compounds (**5a**−**j**, **31a**−**c**, **32**, and **33**) were synthesized in a linear fashion starting from 3-chloro-4-methylaniline. Most of these compounds (e.g., **5a**, **5b, 5g**) were significantly more water-soluble than CB30865 (636 μM for **5a** at pH 6 and 992 μM for **5g** at pH 6). In addition, some of them were up to 6-fold more cytotoxic than CB30865 (e.g., for **5a**, W1L2 $IC_{50} = 0.49 \pm 0.24$ nM) and retained its novel biochemical characteristics.

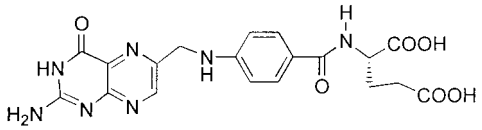
Introduction

4-[*N*-[7-Bromo-2-methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]-*N*-(3-pyridylmethyl)-benzamide (CB30865) is a highly potent cytotoxic agent (W1L2 $IC_{50} = 2.8 \pm 0.50$ nM).[1,2] The compound inhibits isolated mammalian thymidylate synthase (TS), but this inhibition is insufficient to account for its cellular toxicity.[2] Unlike conventional inhibitors of TS, the cytotoxic effects are not reversed in the presence of thymidine/hypoxanthine which indicates a folate-independent locus of action.[1,2] Therefore, the compound has a novel mode of action. CB30865 is lacking the glutamyl residue associated with folic acid; it has been replaced with the 3-(aminomethyl)pyridyl moiety which is believed to play a pivotal role in maintaining the potency of this class of compounds.[1,2] Furthermore, this compound represents a class of agents characterized by some unique and interesting properties. These properties include noncross resistance with other classes of antitumor agents and a delayed, non-phase specific, cell-cycle arrest.[1,2]

The in vivo evaluation of CB30865 was hampered because of its low aqueous solubility (<1 μM at pH 6). This low aqueous solubility of CB30865 initiated a search for more water-soluble analogues to allow the in vivo evaluation of this class of compounds. It was envisaged that aqueous solubility could be increased by the introduction of amino functionalities (e.g., *N*-methylpiperazine) at the 2-position of the quinazolin-4-one ring. Although CB30865 is a 7-bromo derivative, all new analogues contain a chlorine atom at the 7-position because it is considered that a chloro substituent has a lower hydrophobic substituent constant π compared to a bromo substituent. Both CB30865 and its 7-chloro derivative displayed similar inhibitory activities against the W1L2 cell growth.[1b] It was also desirable for the new analogues to retain the cytotoxic potency seen with CB30865, the novel locus of action, and to show non-TS inhibitory activity. The presence of the Me group at the 3-position served to block isolated TS inhibitory activity because it is known that N^3-methylated analogues of quinazoline-based inhibitors of TS displayed poor TS inhibitory activity compared to that of their unsubstituted counterparts.[3] Thus, by utilizing multistep sequences, a variety of compounds have been synthesized (e.g., **5a**, **5b**, **5g**) which have displayed significantly higher aqueous solubilities than CB30865 (636 μM for **5a** at pH 6 and 992 μM for **5g** at pH 6). In addition, some of these compounds were up to 6-fold more cytotoxic than CB30865 and retained its novel characteristics.



CB30865



Folic acid

Chemistry

This class of compounds was synthesized following the synthetic strategy that is outlined in Scheme 1. The

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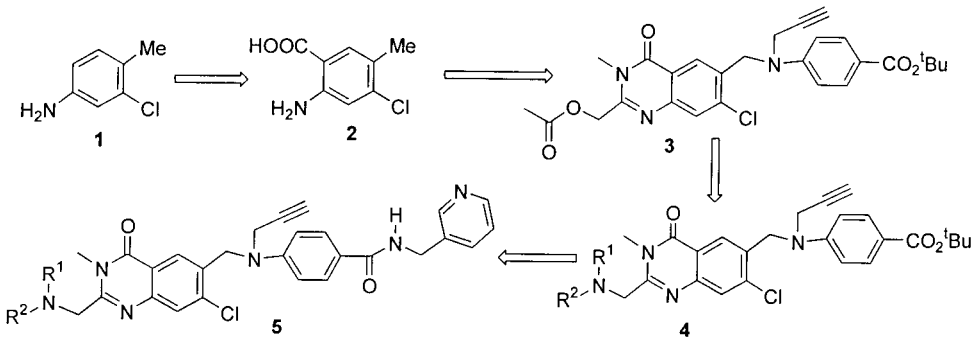
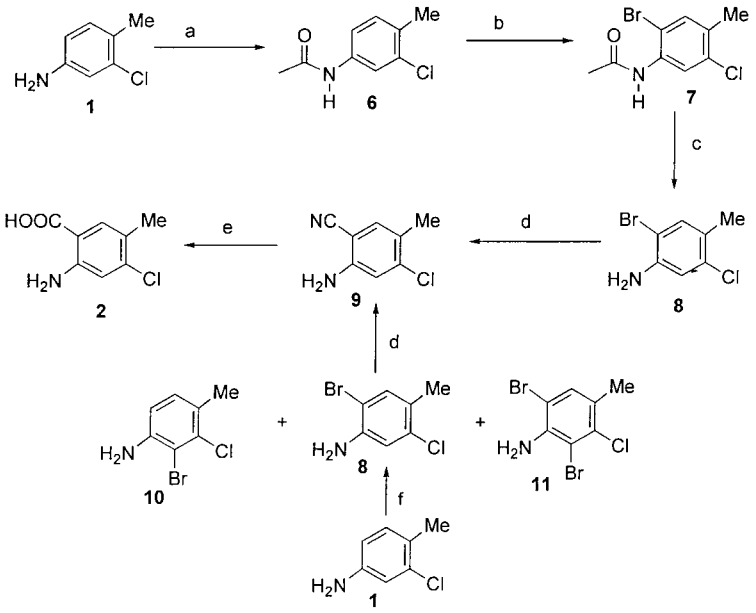
Scheme 1



Scheme 2[a]



[a] Conditions: (a) Ac_2O, pyridine, AcOEt; (b) AcOH/Br_2; (c) AcOH/concd HCl, heat; (d) CuCN, NMP, heat; (e) 30% aq H_2O_2, 30% aq KOH, heat; (f) AcOH, Br_2.

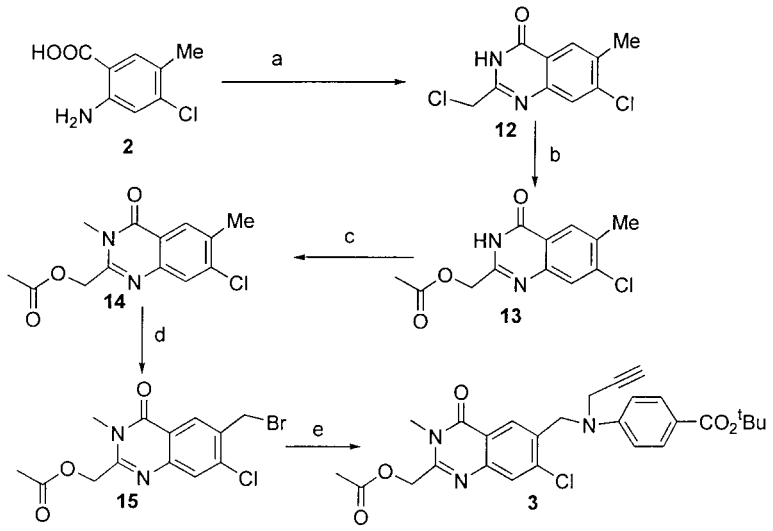
synthesis starts with 3-chloro-4-methylaniline (**1**) which was converted into the anthranilic acid derivative **2**. Because a multistep sequence was utilized, the acid **2** was converted into quinazolin-4-one **3** bearing two differentiable ester groups, therefore allowing the conversion of this molecule into **4**. In the final step, 3-(aminomethyl)pyridine was coupled to the appropriate *p*-aminobenzoic acid derivative to afford the desired products **5a**−**j**.

The synthesis of the first crucial intermediate **2** is shown in Scheme 2. This was synthesized from 3-chloro-4-methylaniline (**1**) via two different routes. In the first route, the amino group of **1** was acetylated using Ac_2O/pyridine in AcOEt, and then bromination of **6** with Br_2/AcOH afforded the bromide **7**. In the next step, the removal of the acetyl group under strong acidic conditions (AcOH, concd HCl, 120 °C) was followed by the displacement of the bromide with the cyanide anion employing CuCN. Hydrolysis of the nitrile **9** under alkaline conditions with the aid of H_2O_2 afforded the anthranilic acid derivative **2**. The intermediate **2** was also prepared from **1** by performing the bromination without protecting the amino functionality of 3-chloro-4-methylaniline (Scheme 2). However, in this case, the bromination results in the formation of two additional products (**10** and **11**) and requires column chromatography to obtain the desired product, making the scale-up of this reaction problematic.

The synthesis of the second key intermediate, compound **3**, is described in Scheme 3. Cyclization of **2** to the 2-chloromethylquinazolin-4-one derivative **12** was effected as previously described.[4] In the next step, the replacement of the Cl atom with an acetoxy group afforded **13**. This nucleophilic displacement proceeded more cleanly and in higher yield when CsOAc was used instead of NaOAc. Treatment of **13** with MeI and NaH in DMF solution afforded the N^3-methyl derivative **14**, and bromination of the latter (NBS/Bz_2O_2/CCl_4) yielded the bromide **15**. Further reaction with *tert*-butyl 4-*N*-(prop-2-ynyl)aminobenzoate in the presence of 2,6-lutidine[4] led to the key intermediate **3**.

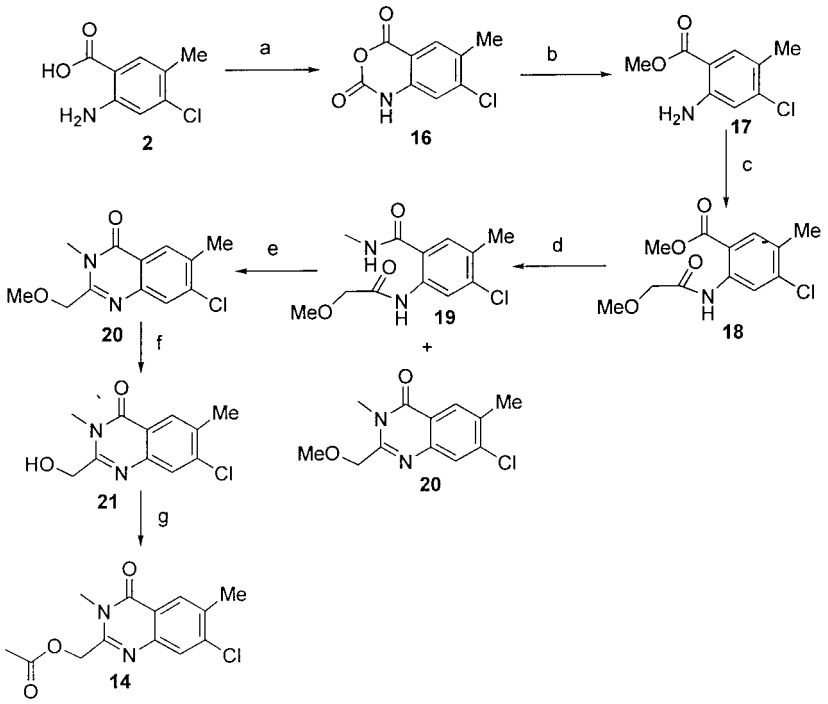
An alternative route to compound **14** was also developed in which the anthranilic acid **2** was converted to its Me ester **17** via the isatoic anhydride **16** (Scheme 4).[5] Compound **17** was then reacted with methoxyacetyl chloride in DMF using pyridine as the base to give **18** which upon treatment with $MeNH_2$ in MeOH/THF gave the *N*-methylbenzamide derivative **19** as the major product and quinazolin-4-one **20** as a byproduct. Cyclization of **19** to the quinazolin-4-one **20** was effected under acidic conditions (AcOH, concd H_2SO_4, 100 °C). The 2-methoxymethyl derivative **20** was next converted

Scheme 3[a]



[a] Conditions: (a) MeOH, Na, $ClCH_2CN$; (b) DMF, CH_3COOCs, heat; (c) MeI, NaH, DMF; (d) NBS, CCl_4, $(PhCO)_2O_2$, heat; (e) *tert*-butyl 4-*N*-(prop-2-ynyl)aminobenzoate, 2,6-lutidine, DMF, heat.

Scheme 4[a]



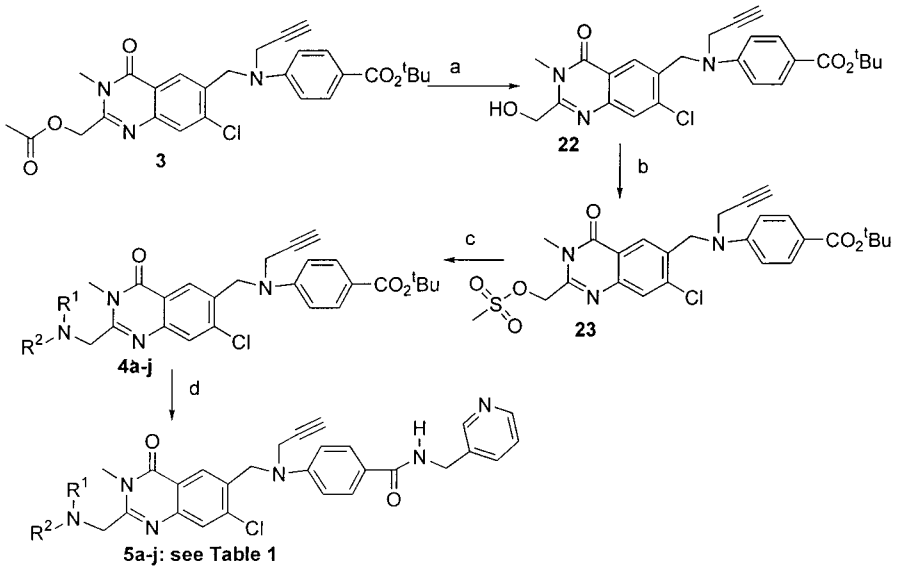
[a] Conditions: (a) triphosgene, THF; (b) MeOH, DMAP, 80 °C; (c) methoxyacetyl chloride, pyridine, DMF; (d) $MeNH_2$, THF/MeOH; (e) AcOH, concd H_2SO_4; (f) 48% HBr, 120 °C; (g) $(CH_3CO)_2O$, Et_3N, DMAP, CH_2Cl_2.

to its 2-hydroxymethyl counterpart **21** upon treatment with 48% HBr at 120 °C.[6] Finally, this compound was quantitatively converted into **14** with Ac_2O, Et_3N, and a catalytic amount of DMAP in CH_2Cl_2.

The final part of the synthesis of compounds **5a**−**j** is shown in Scheme 5. The acetyl group was selectively removed from **3** under alkaline conditions to provide **22**. This compound was then converted into **4a**−**j** via the appropriate derivatization of the hydroxyl functionality (Scheme 5). For example, in the synthesis of **5a**, the 4-methylpiperazin-1-yl moiety was introduced by displacement of the mesylate with 1-methylpiperazine to give **4a**. Finally, the *tert*-butyl ester was removed with TFA, and the resulting benzoic acid derivative was condensed with 3-(aminomethyl)pyridine via a PyBOP carboxyl activation to afford **5a** (Scheme 5).[7]

The synthesis of compounds **31a**−**c** (Figure 1) bearing different N^3 substituents is shown in Scheme 6 in which substituents are introduced in the reverse order relative to Schemes 3−5. First, the amino functionality (i.e., piperidin-1-yl) was introduced at the 2-position by a method analogous to that described for the N^3-methylated derivatives; the N^3 substituent was then introduced by reacting **28** with the appropriate electrophile (e.g., methyl bromoacetate to prepare **29a**) in DMF using NaH as the base. *N*,*N*-Diethylbromoacetamide and 1-(bromoacetyl)piperidine, required for the preparation of **31b** and **31c**, respectively, were prepared fol-

Scheme 5[a]



[a] Conditions: (a) 1 N NaOH, H_2O/THF; (b) $(CH_3SO_2)_2O$, Et_3N, CH_2Cl_2; (c) amine, CH_2Cl_2; (d) (i) TFA, (ii) 3-(aminomethyl)pyridine, PyBOP, DIEA, CH_2Cl_2.

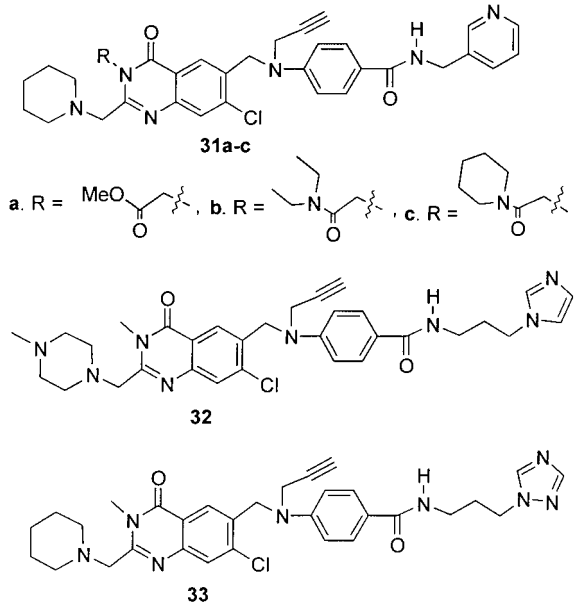


Figure 1.

lowing the literature procedures.[8,9] Alkylation of quinazolin-4-ones has previously been reported to lead to N^3-substituted products or a mixture of N^3 and O^4 products.[10] Only N^3-substituted quinazolin-4-ones were observed in this study, as confirmed by ^{1}H NMR spectroscopy. For compounds **22**, **31a**, and **29c**, the NOESY spectra were obtained. As expected, in each case, the protons of the N^3-alkyl substituent interacted strongly with the 2-CH_2 protons but did not interact with any of the aromatic protons, 5-H or 8-H. It should be noted that **14** was also prepared by the unequivocal route shown in Scheme 4 and that ^{1}H NMR spectra of the product obtained by the two methods were identical.

1-(3-Aminopropyl)-1,2,4-triazole, which was required for the preparation of **33** (Figure 1), was prepared as described by Press et al.[11]

Biological Evaluation

The compounds listed in Tables 1 and 2 were tested as inhibitors of human lymphoblastoid W1L2 cell growth. To confirm that the locus of action is not folate-dependent, all compounds were tested in the W1L2 cell line in the presence of 10 μM thymidine/50 μM hypoxanthine.[2] All compounds were also tested as inhibitors of W1L2:R865 cells, a cell line with resistance to the prototype compound CB30865. Growth inhibition studies were performed as previously described.[2]

In the N^3-methyl series, a variety of substituents at the 2-position were well tolerated with regard to inhibition of W1L2 cell growth (Table 1). Most of these compounds, and in particular **5a**, **5b**, **5c**, **5f**, and **5i**, were more-potent inhibitors of the W1L2 cell growth, compared with the prototype compound CB30865. The most-potent compound was the methylpiperazin-1-yl derivative **5a** (W1L2 $IC_{50} = 0.49 \pm 0.24$ nM) which was 6-fold more potent than CB30865.

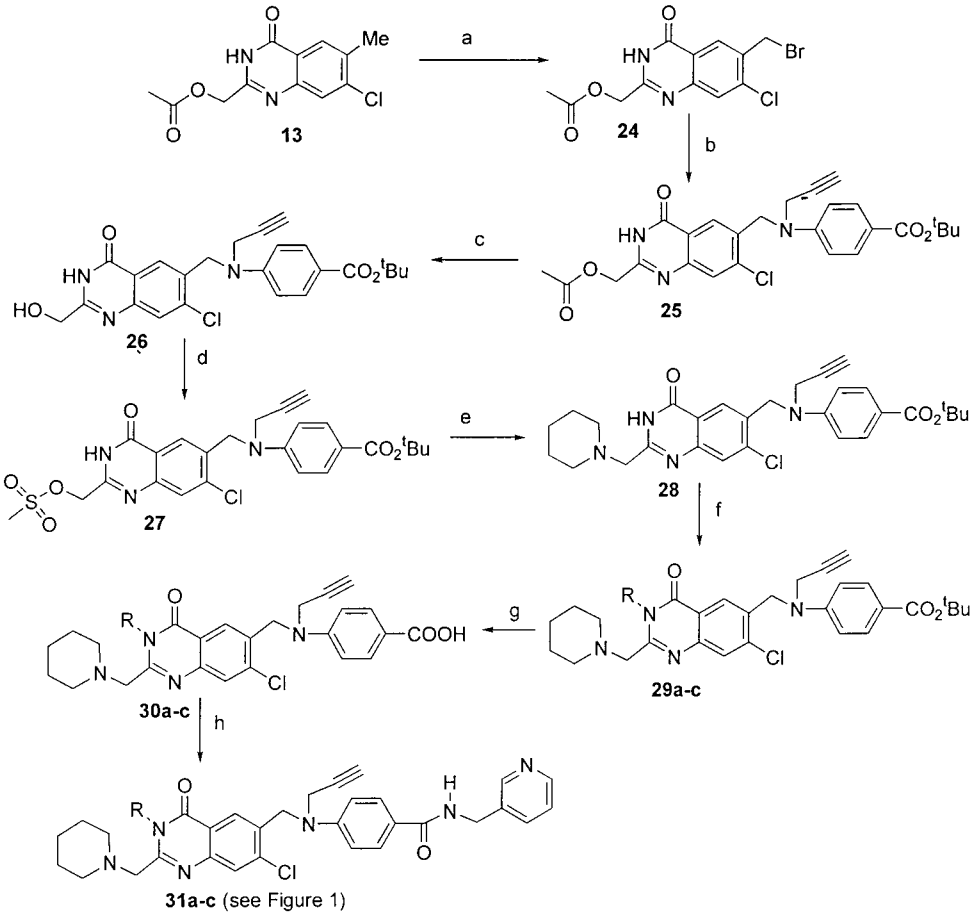
It was established that N^3-methyl derivatives bearing a variety of aminomethyl substituents at the 2-position are potent inhibitors of the W1L2 cell growth; therefore, the SAR with regard to cytotoxicity against this cell line was extended. To this end, bigger substituents than methyl were introduced at the 3-position (compounds **31a**−**c**, Figure 1). In addition, the 3-(aminomethyl)-pyridine moiety in **5a** or **5c** was replaced to give compound **32** or **33**, respectively (Figure 1).

Replacement of the 3-(aminomethyl)pyridine moiety in **5a** by a 1-(3-aminopropyl)imidazole moiety afforded compound **32** which was 4-fold less potent than **5a** (Tables 1 and 2). On the other hand, replacement of the 3-(aminomethyl)pyridyl moiety in **5c** with 1-(3-aminopropyl)-1,2,4-triazole gave compound **33** which was an extremely poor inhibitor of W1L2 cell growth (approximately 1000 times less potent than **5c**).

Of the substituents introduced at the 3-position (compounds **5c**, **31a**−**c**; Tables 1 and 2), the Me substituent is clearly the best for cytotoxic potency.

All the new analogues retained the novel characteristics of the prototype compound CB30865. Their cyto-

Scheme 6[a]



[a] Conditions: (a) NBS, CCl_4, $(PhCO)_2O_2$, heat; (b) *tert*-butyl 4-*N*-(prop-2-ynyl)aminobenzoate, 2,6-lutidine, DMF, heat; (c) 1 N NaOH, H_2O/THF; (d) $(CH_3SO_2)_2O$, Et_3N, DMF; (e) piperidine, DMF; (f) NaH, DMF, electrophile (e.g., $BrCH_2CO_2Me$); (g) TFA; (h) 3-(aminomethyl)pyridine, PyBOP, DIEA, CH_2Cl_2.

toxicity in the W1L2 cell line was retained by the presence of thymidine/hypoxanthine (Tables 1 and 2). All the new compounds were substantially less active in the W1L2:R865 cell line, a cell line made resistant to CB30865. It is interesting to note that the new analogues (except **5h**) were significantly less active than CB30865 in this cell line. This is ascribed to the incorporation of the N^3-methyl group which is reported to reduce TS inhibition.[3] CB30865 inhibits TS at high concentrations in cells, thereby inhibiting the growth of W1L2:R865 cells.[1] Indeed, a selection of compounds in this series (i.e., **5a**, **5b**, and **5c**) inhibited L1210TS very weakly ($IC_{50} > 50\ \mu M$) compared to CB30865 ($IC_{50} = 156$ nM).[2]

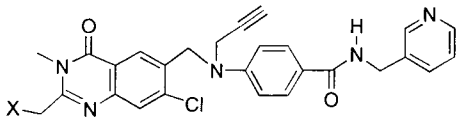
Also, our prime objective in making these compounds more water-soluble than CB30865 has been achieved (Tables 1 and 2). Most compounds, in particular the piperazinyl derivatives **5a**, **5f**, **5g**, and **32**, were significantly more water-soluble than CB30865 (at least 35 times at pH 7.4 and 600 times at pH 6.0; Tables 1 and 2). This property allowed the in vivo evaluation of **5a** in human tumor cell lines grown in the hollow-fiber mouse model. A high level of activity was observed, in particular, in the human CH1 ovarian tumor where ~0.25 mg/kg/day for 3 days almost completely inhibited growth.[13] Recently, **5a** has been shown to weakly inhibit the chymotryptic activity of the 26S proteasome ($IC_{50} \sim 3\ \mu M$), and studies continue to investigate whether this activity contributes to the antitumor effects of the compound.[14]

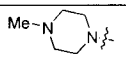
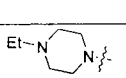
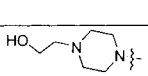
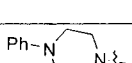
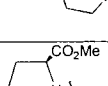
Experimental Section

Thin-layer chromatography (TLC) was performed on precoated sheets of silica $60F_{254}$ (Merck Art 5735). Visualization was achieved by UV or Arnold's base (4,4′-methylene-bis-*N*,*N*-dimethylaniline) reagent.[12] Merck silica 60 (Art 15111) was used in low-pressure column chromatography unless otherwise specified. Petrol refers to light petroleum (bp 60−80 °C). Fast atom bombardment (FAB) mass spectra were determined with a VG ZAB-SE spectrometer. Electrospray ionization (ESI) mass spectra were recorded using a TSQ 700 triple quadruple mass spectrometer (Finnigan MAT) fitted with an electrospray ionization source (Analytica). Proton NMR spectra were recorded using a Bruker AC250 spectrometer. Field strengths are expressed in units of δ (parts per million, ppm) relative to tetramethylsilane, and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; dm, doublet of multiplets; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Elemental analyses were determined by CHN Analysis Ltd., Leicester, U.K.

Determination of Aqueous Solubility. The solubility at pH 6.0 and 7.4 was determined in 10 mM potassium dihydrogen phosphate containing 150 mM sodium chloride and adjusted to either pH 7.4 or 6.0 by the addition of phosphoric acid. The test compound (approximately 2 mg) was added to a 2 mL conical microreaction vial (Supelco, Poole, Dorset, U.K.) and shaken to dissolve the contents. The vial was wrapped in foil and placed in a shaking water bath at 25 °C for 18 h. At this time, the shaker was stopped, and the contents were left to settle for 30 min. The pH was remeasured, and the pH was

Table 1. Cell Growth Inhibition and Aqueous Solubility[a]



Compnd	X	W1L2 IC_{50} (nM)	W1L2+dThd/HX IC_{50} (nM)	W1L2:R865 IC_{50} (nM)	Solubility (μM) pH6.0	pH7.4
CB30865	see text	2.8±0.50	2.2±0.82	610±82	<1	2.3
5a		0.49±0.24	0.32, 0.58	13000±4500	636	146
5b		0.71±0.076	0.73±0.050	>50000	Not determined	
5c		0.80, 0.80	0.78	14000	Not detected	
5d		2.0±0.36	2.0	19000	3	2
5e		0.70, 0.74	0.76, 0.70	24000	Not determined	
5f		0.78. 0.80	0.78, 0.76	22000, 20000	1765	286
5g		7.0, 1.9	7.1, 2.0	22000, 19000	992	75
5h		7.2, 2.8	5.4, 3.1	940, 700	23	5
5i		0.72, 0.74	0.70, 0.72	18000	2	0.5
5j		18, 6.6	14, 7.6	24000, 19000	Not determined	

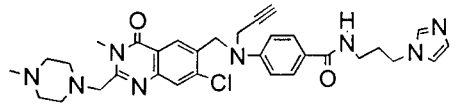
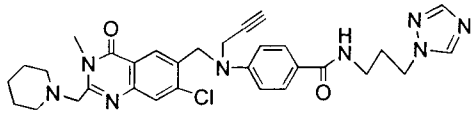
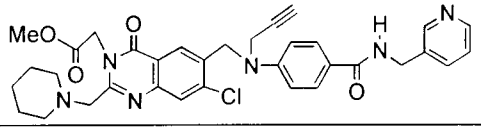
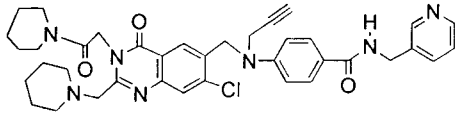
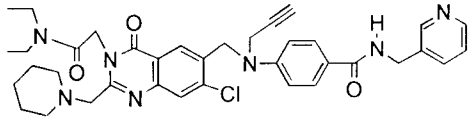
[a] Cell growth inhibition was measured using cell counting as described previously.[2] The results are given as the mean ± SD or as individual results. The solubility at pH 6.0 and 7.4 was determined in 10 mM potassium dihydrogen phosphate containing 150 mM sodium chloride and adjusted to either pH 7.4 or 6.0 by the addition of phosphoric acid (Experimental Section).

adjusted with dilute phosphoric acid or potassium hydroxide solution if the value was outside the pH limit of ±0.2. When this adjustment was necessary, the vial and contents were allowed to reequilibrate for a further 60 min. The vial was centrifuged at 1000*g* for 15 min, and the contents were filtered through a 0.2 μm Supelco IsoDisc N, Nylon membrane filter. A 100 μL aliquot was diluted to a 1:1 ratio with HPLC-grade MeCN and was subjected to HPLC analysis. The HPLC conditions consisted of a 150 × 4.6 mm Supelcosil RP18 column (Supelco) and a mobile phase consisting of 50 mM ammonium acetate and sufficient HPLC-grade MeCN to produce a retention time between 5 and 10 min. The flow rate was 1.25 mL/min; the column temperature was 45 °C, and the injection volume was 25 μL. The concentration of the test compound was determined using UV detection at 295 nm and was quantified with reference to a 3-point standard curve. The range for this curve was established by calculating the concentration of the solubility test solution using UV spectrophotometry and the previously measured extinction coefficient. Calibration solutions for the standard curve were prepared at 4, 2, and 0.5 times this estimated concentration.

4-Acetamido-2-chlorotoluene (6). Acetic anhydride (78 mL, 0.825 mol) was added in portions (using a dropping funnel) during a 45 min period to a stirred, ice bath-cooled solution of 3-chloro-4-methylaniline (106.2 g, 0.75 mol) in EtOAc (550 mL, dried over $MgSO_4$ prior to use) and anhydrous pyridine (66.6 mL, 0.825 mol) under argon. During the reaction, the temperature of the reaction mixture varied between 10 and 20 °C. The mixture was stirred for 20 min; the ice/water bath was then removed, and the reaction mixture was stirred for 18 h at room temperature. The solvents were removed in vacuo, and the light brown solid residue was triturated with Et_2O (350 mL) and left to stand in a refrigerator overnight. The solid was collected by filtration, washed with cold Et_2O (100 mL) and hexanes (100 mL), and dried over P_2O_5 to afford a white solid (90 g). The filtrate was concentrated in vacuo and triturated with Et_2O to afford an additional 28.1 g of the product: total yield 118.1 g (86%); mp 105−106 °C; ^1H NMR ($CDCl_3$) 2.17, 2.32 (2 × s, 6H, 4-CH_3, CH_3CO), 7.14 (d, J = 8.22 Hz, 1H, 6-H), 7.25 (2 × dd, J = 1.9, 6.5 Hz, 1H, 5-H), 7.58 (d, J = 1.8 Hz, 1H, 3-H); MS (FAB, *m/z*) 184, and 186 [$(M + H)^+$; 100 and 30%, respectively; Cl isotopic pattern]. Anal. ($C_9H_{10}ClNO$) C, H, N, Cl.

4-Acetamido-5-bromo-2-chlorotoluene (7). To a solution of 4-acetamido-2-chlorotoluene (89.2 g, 0.486 mol) in glacial AcOH (480 mL) that was stirred with an overhead mechanical stirrer under argon was dropwise added bromine (28.5 mL) during a period of 2 h while the temperature of the reaction mixture was kept below 15 °C by using an ice bath. The mixture was stirred for a further 1.5 h after the addition of bromine, under an argon atmosphere. The brownish reaction mixture was then poured into ice/water (1.8 L), with

Table 2. Cell Growth Inhibition and Aqueous Solubility[a]

compd	Structure	W1L2 IC_{50} (nM	W1L2+dThd/HX IC_{50} (nM)	W1L2:R865 IC_{50} (nM)	Solubility pH6.0	pH7.4
32		2.1, 2.2	2.3, 2.2	18000, 14000	934	96
33		840, 820	800, 840	20000, 31000	80	6
31a		11, 14	9.6, 17	23000, 31000	Not determined	
31b		8.4	7.4	>50000	Not determined	
31c		9.4, 7.4	8.4, 7.4	19000, 28000	2	2

[a] Cell growth inhibition was measured using cell counting as described previously.[2] The results are given as the mean ± SD or as individual results. The solubility at pH 6.0 and 7.4 was determined in 10 mM potassium dihydrogen phosphate containing 150 mM sodium chloride and adjusted to either pH 7.4 or 6.0 by the addition of phosphoric acid (Experimental Section).

the aid of H_2O (1 L), washed with H_2O (6 L), and dried in vacuo over P_2O_5. Recrystallization from MeCN afforded **7** as white crystals (61.5 g, 48%): mp 154−155 °C; ^{1}H NMR ($CDCl_3$) 2.24, 2.32 (2 × s, 6H, 2 × CH_3), 7.40, 7.73 (2 × s, 2H, 3-H, 6-H), 7.49 (br s, 1H, CONH); MS (FAB, *m*/*z*) 262, 264, and 266 [$(M+H)^+$; 80, 100, and 25%, respectively; BrCl isotopic pattern]. Anal. ($C_9H_9BrClNO$) C, H, N, Cl, Br.

2-Bromo-5-chloro-4-methylaniline (8). To a solution of 3-chloro-4-methylaniline (10.0 g, 70.6 mmol) in Et_2O/AcOH (v/v, 1/1, 350 mL) which was cooled in an ice bath was added dropwise bromine (4 mL) over a 35 min period under an argon atmosphere while the temperature of the reaction mixture was kept below 5 °C. The mixture was stirred for another 10 min after the addition of bromine, and then the yellow reaction mixture was partitioned between CH_2Cl_2 (250 mL) and brine (200 mL). The organic layer was washed with brine (200 mL), dried (Na_2SO_4), and concentrated in vacuo to an oily residue. This was redissolved in CH_2Cl_2 (200 mL); the solution was washed with saturated aqueous $NaHCO_3$ (3 × 200 mL; **caution**: gas is evolved), dried (Na_2SO_4), and concentrated in vacuo to leave a brown, wet solid. Purification by column chromatography using a 25−30% gradient of CH_2Cl_2 in hexanes gave the following compounds in order of elution.

11: 4.63 g; mp 77−78 °C. **8**: 5.32 g (34%); mp 90 °C; ^{1}H NMR ($CDCl_3$) 2.23 (s, 3H, 4-CH_3), 3.99 (br s, 2H, NH_2), 6.78 (s, 1H, 6-H), 7.26 (s, 1H, 3-H); MS (FAB, *m*/*z*) 219, 221, 223 [$(M + H)^+$; BrCl isotopic pattern]. Anal. (C_7H_7BrClN) C, H, N, Cl, Br. **10**: 1.22 g; mp 48−56 °C.

Compound **8** (2-bromo-5-chloro-4-methylaniline) was also prepared as follows:

A solution of **7** (64 g, 0.245 mol) in glacial AcOH (48 mL) and concentrated HCl (96 mL) was heated at 118 °C for 24 h. The reaction mixture was allowed to cool to room temperature, diluted with water (200 mL), and cooled in an ice bath, and the pH was adjusted to 5 with an aqueous solution of NaOH (50% w/v). The precipitate was collected by filtration, washed with water, and dried in vacuo over P_2O_5 to afford a white solid (50.7 g, 94%, mp 90 °C).

2-Cyano-5-chloro-4-methylaniline (9). To a solution of **8** (13.0 g, 58.96 mmol) in *N*-methylpyrrolidinone (100 mL) was added CuCN (10.56 g, 117.9 mmol). The reaction mixture was placed in an oil bath preheated to 163 °C and was stirred at this temperature for 2 h. The reaction mixture was allowed to cool to room temperature, and then poured into ice/water (300 mL) and aqueous NH_3 (90 mL). The brown precipitate was collected by filtration, washed with water (150 mL), and dissolved in CH_2Cl_2, and the insoluble material was removed by filtration. The filtrate was washed with brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification, on gradient elution with CH_2Cl_2 in petroleum ether to 60−80 °C (65−95%), afforded a white solid (6.52 g): mp 180 °C; ^{1}H NMR (DMSO-d_6) 2.14 (s, 3H, 4-CH_3), 6.11 (s, 2H, NH_2), 6.86 (s, 1H, 6-H), 7.39 (s, 1H, 3-H); MS (FAB, *m*/*z*) 166 and 168 [$(M + H)^+$; 90 and 40%, respectively; Cl isotopic pattern]; FAB-HRMS measured 166.0307, calcd for $C_8H_8ClN_2$ [$(M + H)^+$] 166.0298.

2-Amino-4-chloro-5-methylbenzoic Acid (2). A mixture of 5-chloro-2-cyano-4-methylaniline (4.0 g, 0.024 mol), 30% aqueous KOH solution (56 mL), and 30% H_2O_2 (4 mL) was placed in an oil bath preheated to 130 °C, and then stirred at this temperature for 2 h (a clear solution was obtained after 1.5 h). The clear solution was then allowed to cool to room temperature, diluted with H_2O (200 mL), acidified with 3 N HCl to pH ∼5.50, and allowed to stand at room temperature

for several hours. The off-white solid was collected by filtration, washed with H_2O, and dried in vacuo over P_2O_5 (4.13 g, 93%): mp 212−215 °C; ^{1}H NMR (DMSO-d_6) 2.16 (s, 3H, CH_3), 6.83, 7.62 (2 × s, 2H, 3-H, 6-H), 8.50 (br s, 2H, NH_2); MS (FAB, *m*/*z*) 188, 186 [(M + H)$^+$]; FAB-HRMS measured 185.0256, calcd for $C_8H_8ClNO_2$ (M^+) 185.0244. Anal. ($C_8H_8ClNO_2$) H, N, Cl; C: calcd 51.77, found 50.67.

7-Chloro-2-chloromethyl-6-methyl-3,4-dihydroquinazolin-4-one (12). To a flask containing sodium (36 mg) was added anhydrous MeOH (5 mL). Chloroacetonitrile (0.520 g, 6.9 mmol) was then added, and the clear solution was stirred at room temperature for 30 min under argon. A solution of **2** (1.13 g, 6.0 mmol) in anhydrous MeOH (25 mL) was then added with a syringe via a rubber septum. After the reaction mixture was stirred at room temperature for 2 h under argon, the flask was fitted with a condenser and placed in an oil bath preheated to 80 °C. The mixture was stirred at this temperature for 2 h under argon, and then the reaction mixture was allowed to cool to room temperature. The precipitate was collected by filtration, washed with MeOH (10 mL) and H_2O (10 mL), and dried in vacuo over P_2O_5 to afford a gray solid (1.0 g, 69%): mp 287−290 °C; ^{1}H NMR (DMSO-d_6) 2.47 (s, 3H, 6-CH_3), 4.53 (s, 2H, CH_2Cl), 7.75, 8.08 (2 × s, 2H, 5-H and 8-H), 12.60 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 243, 244, 245 [(M + H)$^+$]. Anal. ($C_{10}H_8Cl_2N_2O$) C, H, N, Cl.

2-Acetoxymethyl-7-chloro-6-methyl-3,4-dihydroquinazolin-4-one (13). A mixture of **12** (0.500 g, 2.06 mmol), anhydrous DMF (14 mL), and cesium acetate (1.58 g, 8.24 mmol) was placed in an oil bath preheated to 85 °C, and then stirred at this temperature for 2 h and 15 min under argon. The reaction mixture was then allowed to cool to room temperature, and the solvent was removed in vacuo. The residue was treated with hexanes (20 mL), washed with hexanes (20 mL) and H_2O, and dried in vacuo over P_2O_5 (0.476 g, 87%): mp 220−225 °C, ^{1}H NMR (DMSO-d_6) 2.14 (s, 3H, CH_3CO), 2.45 (s, 3H, 6-CH_3), 4.95 (s, 2H, 2-CH_2O), 7.70, 8.06 (2 × s, 2H, 5-H and 8-H), 12.44 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 267 [(M + H)$^+$]; FAB-HRMS measured 267.0520, calcd for $C_{12}H_{12}ClN_2O_3$ [(M + H)$^+$] 267.0536.

2-Acetoxymethyl-7-chloro-3,6-dimethyl-3,4-dihydroquinazolin-4-one (14). Method A. To a suspension of **13** (0.428 g, 1.6 mmol) in anhydrous DMF (13 mL) was added NaH (60% dispersion, 0.070 g, 1.76 mmol) under argon. The mixture was stirred at room temperature for 1 min, and then MeI (0.20 mL, 3.2 mmol) was added into the reaction mixture with a syringe via a septum. The mixture was stirred at room temperature for 1 h, and the reaction mixture was then partitioned between AcOEt (130 mL) and brine (80 mL). The organic layer was washed with brine (80 mL), dried (Na_2SO_4), and concentrated in vacuo to leave an orange residue. This orange residue was dissolved in CH_2Cl_2, and to this solution was added silica gel (Art 7734, 1.7 g). The solvent was removed in vacuo, and the orange free-running powder was placed on a silica gel column made up in 5% AcOEt in CH_2Cl_2. The column was eluted with 5% AcOEt in CH_2Cl_2 to afford a pale yellow solid (0.300 g, 67%): mp 110−112 °C; ^{1}H NMR (DMSO-d_6) 2.17 (s, 3H, CH_3CO), 2.47 (s, 3H, 6-CH_3), 3.54 (s, 3H, N^3-CH_3), 5.23 (s, 2H, 2-CH_2O), 7.71, 8.09 (2 × s, 2H, 5-H, 8-H); MS (FAB, *m*/*z*) 281 and 283 [(M + H)$^+$; 100 and 25%, respectively; Cl isotopic pattern]. Anal. ($C_{13}H_{13}ClN_2O_3$) C, H, N, Cl.

Method B. This compound was also prepared by acetylating compound **21**. To a mixture of **21** (0.051 g, 0.21 mmol) and anhydrous CH_2Cl_2 (1.5 mL) was added DMAP (catalytic amount, 0.002 g), followed by a solution of Et_3N (0.028 g, 0.28 mmol) in anhydrous CH_2Cl_2 (0.3 mL) and a solution of acetic anhydride (0.028 g, 0.28 mmol) in anhydrous CH_2Cl_2 (0.3 mmol). The clear solution was stirred at room temperature for 30 min, and then it was partitioned between AcOEt (30 mL) and saturated aqueous $NaHCO_3$ (30 mL). The organic layer was washed with more saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo to give the title compound **14** as a white solid (0.058 g, 98%).

2-Acetoxymethyl-6-bromomethyl-7-chloro-3-methyl-3,4-dihydroquinazolin-4-one (15). To a nearly clear solution of **14** (2.85 g, 10.16 mmol) in anhydrous CCl_4 (60 mL) under argon was added NBS (1.99 g, 11.17 mmol) followed by dibenzoyl peroxide (25 mg). The reaction flask was then fitted with a condenser and placed in an oil bath preheated to 85 °C and illuminated with two 60 W bulbs. The mixture was stirred at this temperature for 3 h and 50 min, and then the reaction mixture was allowed to cool to room temperature. The white precipitate was filtered off and washed with CH_2Cl_2, and the filtrate was concentrated in vacuo to leave a white solid. This was partitioned between AcOEt (200 mL)/CH_2Cl_2 (25 mL) and brine (100 mL). The organic layer was washed with brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. The white solid was dissolved in CH_2Cl_2, and to this solution was added silica gel (Art 7734, 3.5 g). The solvent was removed in vacuo, and the white free-running powder was placed on a silica gel column made up in 5% AcOEt in CH_2Cl_2. The column was eluted with a gradient of AcOEt in CH_2Cl_2 (5−10%). Fractions found to be pure by TLC were combined and evaporated to obtain a solid which was triturated with AcOEt/hexanes (v/v, 4/6, 20 mL). The white solid was collected by filtration and dried in vacuo (1.83 g, 51%): mp 183−186 °C; ^{1}H NMR (DMSO-d_6) 2.18 (s, 3H, $COCH_3$), 3.52 (s, 3H, N^3-Me), 4.92 (s, 2H, CH_2Br), 5.25 (s, 2H, 2-CH_2O), 7.78, 8.39 (2 × s, 2H, 5-H, 8-H); MS (FAB, *m*/*z*) 363, 361 and 359 [(M + H)$^+$; 30, 100, 80%, respectively; BrCl isotopic pattern]. Anal. ($C_{13}H_{12}BrClN_2O_3$) C, H, N, Br, Cl.

***tert*-Butyl 4-[*N*-[2-Acetoxymethyl-7-chloro-3-methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)-amino]benzoate (3).** A flask containing **15** (1.75 g, 4.88 mmol), anhydrous DMF (30 mL), *tert*-butyl 4-*N*-(prop-2-ynyl)-aminobenzoate (1.35 g, 5.86 mmol), and 2,6-lutidine (1.38 g, 12.89 mmol) was fitted with a condenser and placed in an oil bath preheated to 120 °C, and then stirred at this temperature for 5.5 h under argon. Next, the solvent was removed in vacuo, and the brown residue was partitioned between AcOEt (350 mL) and brine (120 mL). The organic layer was washed with more dilute brine (120 mL), dried (Na_2SO_4), and concentrated in vacuo. The brown residue was purified by column chromatography using CH_2Cl_2/AcOEt/petroleum ether (v/v/v, 4/3/3) at 60−80 °C as eluant. Fractions pure by TLC and not positive to Epstein's spray were combined and concentrated in vacuo to give 1.48 g of the desired product as a white solid. Fractions positive to Epstein's spray (contaminated with a small amount of bromide) were combined and concentrated in vacuo, and the residue was triturated with hexanes/AcOEt (v/v, 7/3, ∼10 mL) and dried in vacuo over P_2O_5 to afford an additional 0.340 g of the product (total yield 1.82 g, 73%): mp 165−167 °C; ^{1}H NMR (DMSO-d_6) 1.50 (s, 9H, Bu^t), 2.17 (s, 3H, CH_3CO), 3.46 (s, 3H, N^3-Me), 4.39 (s, 2H, $CH_2C{\equiv}C$), 4.80 (s, 2H, 6-CH_2), 5.22 (s, 2H, 2-CH_2), 6.79 (d, J = 8.7 Hz, 2H, 3′,5′-ArH), 7.73 (d, J = 8.5 Hz, 2H, 2′,6′-ArH), 7.79, 7.91 (2 × s, 5-H, 8-H); MS (FAB, *m*/*z*) 509 and 511 (M^+; 70 and 30%, respectively; Cl isotopic pattern); FAB-HRMS measured 509.1750, calcd for $C_{27}H_{28}ClN_3O_5$ (M^+) 509.1717. Anal. ($C_{27}H_{28}ClN_3O_5{\cdot}0.25H_2O$) C, H, N, Cl.

7-Chloro-6-methyl-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (16). To a solution of **2** (2.50 g, 13.47 mmol) in anhydrous THF (55 mL) was added triphosgene (1.36 g, 4.55 mmol). The reaction mixture was stirred at room temperature for 6 h under argon, and then it was left to stand in a refrigerator overnight. The precipitate was collected by filtration, washed with Et_2O, and dried in vacuo over P_2O_5 to afford 1.93 g of the title compound **16** as a white solid. The filtrate was concentrated in vacuo. Trituration of the residue with Et_2O afforded an additional 0.78 g of the product: total yield 97%; mp 257−260 °C; ^{1}H NMR (DMSO-d_6) 2.34 (s, 3H, CH_3), 7.15, 7.90 (2 × s, 2H, 5-H, 8-H). This material was used in the next experiment without any further purification.

Methyl 2-Amino-4-chloro-5-methylbenzoate (17). To a mixture of **16** (1.30 g, 6.15 mmol) in anhydrous MeOH (45 mL) was added DMAP (0.070 g). The reaction mixture was then placed in an oil bath preheated to 80 °C and stirred at this

temperature for 3 h under argon. The solvent was then removed in vacuo, and the residue was partitioned between AcOEt (250 mL) and 0.1 M HCl (100 mL). The organic layer was washed with 0.1 M HCl (2 × 100 mL), dried (Na_2SO_4), and concentrated in vacuo to give the title compound **17** as a white solid: 1.1 g, 90%; mp 70−71 °C; ^{1}H NMR (DMSO-d_6) 2.16 (s, 3H, CH_3), 3.78 (s, 3H, CO_2Me), 6.87, 7.63 (2 × s, 2H, 3-H, 6-H), 6.60 (br s, 2H, NH_2); MS (ESI, *m*/*z*) 200 and 202 [(M + H)$^+$, 100 and 35%, respectively; Cl isotopic pattern]. Anal. ($C_9H_{10}ClNO_2$) H, N; C: calcd 54.15, found 53.45.

Methyl 2-Methoxyacetamido-4-chloro-5-methylbenzoate (18). To a solution of **17** (1.03 g, 5.18 mmol) in anhydrous DMF (13 mL) was added methoxyacetyl chloride (1.24 g, 11.40 mmol) followed by pyridine (2.10 mL, 25.9 mmol). The reaction mixture was stirred at room temperature for 2 h under argon, and then it was partitioned between AcOEt (250 mL) and 1 N HCl (100 mL). The organic layer was washed with 1 N HCl (2 × 80 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with 35% AcOEt in hexanes, afforded the title compound **18** as a white solid (1.26 g, 90%): mp 120−121 °C; ^{1}H NMR (DMSO-d_6) 2.33 (s, 3H, 5-CH_3), 3.45 (s, 3H, CH_2OMe), 3.88 (s, 3H, CO_2Me), 4.05 (s, 2H, C*H*$_2$OMe), 7.97, 8.72 (2 × s, 2H, 3-H, 6-H), 11.42 (s, 1H, CONH); MS (ESI, *m*/*z*) 272 and 274 [(M + H)$^+$; 100 and 32%, respectively; Cl isotopic pattern]. Anal. ($C_{12}H_{14}ClNO_4$) C, H, N, Cl.

2-Methoxyacetamido-4-chloro-5-methyl *N*-Methylbenzamide (19). To a mixture of **18** (1.18 g, 4.4 mmol) and $MeNH_2$ (2 M solution in MeOH, 50 mL) was added more $MeNH_2$ (2 M solution in THF, 25 mL). The clear solution was stirred at room temperature for 18 h, and then more $MeNH_2$ (2 M solution in MeOH, 18 mL; 2 M solution in THF, 8 mL) was added into the reaction mixture. The mixture was stirred at room temperature for another 8 h, and then the solvents were removed in vacuo. The residue was triturated with Et_2O to give 0.375 g of the title compound **19** as a white solid. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (eluant 35% AcOEt in hexanes) to give the following compounds in order of elution: (**a**) 0.120 g of the starting material **18**; (**b**) 0.230 g of compound **20**; and (**c**) an additional 0.240 g of the title compound **19**. Yield for **19**: 52%; mp 210 °C; ^{1}H NMR (DMSO-d_6) 2.32 (s, 3H, 5-CH_3), 2.78 (d, J = 4.5 Hz, 3H, NHMe), 3.40 (s, 3H, CH_2OMe), 3.99 (s, 2H, C*H*$_2$OMe), 7.73, 8.63 (2 × s, 2H, 3-H, 6-H), 8.72 (m, 1H, CON*H*Me); MS (ESI, *m*/*z*) 271 and 273 [(M + H)$^+$; 100 and 40%, respectively; Cl isotopic pattern]. Anal. ($C_{12}H_{15}ClN_2O_3$) H, N;C: calcd 53.24, found 52.67.

2-Methoxymethyl-7-chloro-3,6-dimethyl-3,4-dihydroquinazolin-4-one (20). To a nearly clear solution of **19** (0.320 g, 1.18 mmol) in AcOH (30 mL) was added concd H_2SO_4 (1.1 mL). The reaction mixture was placed in an oil bath preheated to 100 °C. The clear solution was heated at this temperature for 6.5 h, and then it was concentrated in vacuo to a volume of ∼10 mL. This was diluted with H_2O (45 mL), and the pH was adjusted to ∼4 with solid Na_2CO_3. CH_2Cl_2 (150 mL) was then added into the mixture. The two layers were separated, and the aqueous layer was extracted with more CH_2Cl_2 (2 × 30 mL). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo. Purification of the residue by column chromatography, on elution with 20% AcOEt in CH_2Cl_2, afforded the title compound **20** as a white solid (0.243 g, 87%): mp 128 °C; ^{1}H NMR (DMSO-d_6) 2.50 (s, 3H, 6-CH_3), 3.41 (s, 3H, CH_2OMe), 3.58 (s, 3H, N^3-Me), 4.59 (s, 2H, C*H*$_2$OMe), 7.78, 8.13 (2 × s, 2H, 5-H, 8-H); MS (ESI, *m*/*z*) 253 and 255 [(M + H)$^+$; 100 and 40%, respectively; Cl isotopic pattern]. Anal. ($C_{12}H_{13}ClN_2O_2$) H, N, Cl; C: calcd 57.04, found 56.62.

2-Hydroxymethyl-7-chloro-3,6-dimethyl-3,4-dihydroquinazolin-4-one (21). A mixture of **20** (0.118 g, 0.47 mmol) and 48% aqueous HBr (10 mL) was placed in an oil bath preheated to 120 °C. The clear solution was stirred at this temperature for 7 h; it was then allowed to cool to room temperature and diluted with H_2O (5 mL), and the pH was adjusted to ∼4 with NaOH pellets. The white precipitate was collected by filtration, washed with H_2O, and dried in vacuo over P_2O_5. Purification by column chromatography, on elution with 20% AcOEt in CH_2Cl_2, afforded the title compound **21** as a white solid (0.086 g, 76%): mp 175 °C; ^{1}H NMR (DMSO-d_6) 2.50 (s, 3H, 6-CH_3), 3.60 (s, 3H, N^3-Me), 4.61 (d, J = 5.67 Hz, 2H, C*H*$_2$OH), 5.66 (t, J = 6.13 Hz, 1H, OH), 7.76, 8.13 (2 × s, 2H, 5-H, 8-H); MS (ESI, *m*/*z*) 239 and 241 [(M + H)$^+$; 100 and 40%, respectively; Cl isotopic pattern]. Anal. ($C_{11}H_{11}ClN_2O_2$) C, H, N.

***tert*-Butyl 4-[*N*-[7-Chloro-2-hydroxymethyl-3-methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (22).** To a solution of **3** (0.47 g, 0.92 mmol) in THF (18 mL) was slowly added 1 N aqueous NaOH (1.84 mL, 1.84 mmol) followed by H_2O (1.5 mL). The slightly cloudy solution was stirred at room temperature for 1 h; the solvent was removed in vacuo, and the residue was treated with H_2O (35 mL). The pH was adjusted to 4.5 with 1 N HCl, and the mixture was extracted with AcOEt (3 × 60 mL). The organics were combined, dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with 50% AcOEt in CH_2Cl_2, afforded a white solid which was reprecipitated from CH_2Cl_2 (minimum amount)/hexanes. The solid was collected by filtration and dried in vacuo over P_2O_5 (0.345 g, 80%): mp 109−111 °C; ^{1}H NMR (DMSO-d_6) 1.49 (s, 9H, CO_2Bu^t), 3.50 (s, 3H, N^3-H), 3.25 (s, poorly resolved triplet; 1H, C≡CH), 4.40 (s, 2H, CH_2C≡C), 4.57 (d, J = 5.70 Hz, 2H, 2-CH_2OH), 4.80 (s, 2H, 6-CH_2), 5.67 (t, J = 6.4 Hz, 1H, CH_2OH), 6.78 (d, J = 8.80 Hz, 2H, 2′,6′-ArH), 7.82, 7.87 (2 × s, 2H, 5-H, 8-H); MS (FAB, *m*/*z*) 467 and 469 (M$^+$; 95 and 45%, respectively; Cl isotopic pattern). Anal. ($C_{25}H_{26}ClN_3O_4$) C, H, N, Cl.

***tert*-Butyl 4-[*N*-[7-Chloro-2-methanesulfonyloxymethyl-3-methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (23).** To a stirred, ice bath-cooled solution of **22** (0.200 g, 0.43 mmol) in anhydrous CH_2Cl_2 (5 mL) under argon was added Et_3N (0.152 g, 1.5 mmol) followed by methanesulfonic anhydride (0.120 g, 0.69 mmol; added in one portion). After 10 min, the ice bath was removed and stirring was continued for 45 min; TLC (40% AcOEt in CH_2Cl_2) indicated a complete reaction. The reaction mixture was then diluted with AcOEt (200 mL), and the solution was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with 40% AcOEt in CH_2Cl_2, afforded a white solid which was dried in vacuo over P_2O_5 (0.221 g, 94%): mp 204−205 °C; ^{1}H NMR (DMSO-d_6) 1.50 (s, 9H, CO_2Bu^t), 3.17 (s, poorly resolved triplet; 1H, C≡CH), 3.20 (s, 3H, SO_2Me), 3.49 (s, 3H, N^3-Me), 4.39 (d, J = 2.2 Hz, 2H, CH_2C≡C), 4.82 (s, 2H, 6-CH_2), 5.41 (s, 2H, 2-CH_2), 6.79 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.73 (d, J = 8.9 Hz, 2H, 2′,6′-ArH), 7.88, 7.94 (2 × s, 2H, 5-H, 8-H); MS (FAB, *m*/*z*) 546 and 548 [(M + H)$^+$; 95 and 44%, respectively; Cl isotopic pattern]. Anal. ($C_{26}H_{28}ClN_3O_6S$) C, H, N.

***tert*-Butyl 4-[*N*-[7-Chloro-3-methyl-2-(4-methyl-piperazin-1-yl)methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (4a).** To a solution of **23** (0.205 g, 0.38 mmol) in anhydrous CH_2Cl_2 (8 mL) under argon was slowly added 1-methylpiperazine (0.376 g, 3.76 mmol). The mixture was stirred under argon for 2.5 h at room temperature;the reaction mixture was then diluted with AcOEt (200 mL), washed with 6% Na_2CO_3 (w/v solution, 2 × 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with 5% MeOH in CH_2Cl_2, afforded a white solid (0.159 g, 77%): mp 136−138 °C; ^{1}H NMR (DMSO-d_6) 1.50 (s, 9H, Bu^t), 2.14 (s, 3H, N-Me piperazine), 2.29 (br s) and 2.50 (br s obscured, 8H, N(CH_2CH_2)$_2$), 3.60 (s, 3H, N^3-Me), 3.62 (s, 2H, 2-CH_2), 4.38 (d, J = 1.1 Hz, 2H, CH_2C≡C), 4.80 (s, 2H, 6-CH_2), 6.79 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.73 (d, J = 8.9 Hz, 2′,6′-ArH), 7.91, 7.79 (2 × s, 2H, 5-H, 8-H); MS (FAB, *m*/*z*) 550 and 552 [(M + H)$^+$; 100 and 35%, respectively; Cl isotopic pattern]. Anal. ($C_{30}H_{36}ClN_5O_3$) C, H, N, Cl.

4-[*N*-[7-Chloro-3-methyl-2-(4-methyl-piperazin-1-yl)-methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-

2-ynyl)amino]-*N*-(3-pyridylmethyl)benzamide (5a). A solution of **4a** (0.094 g, 0.17 mmol) in CH_2Cl_2 (1.2 mL) and TFA (1.6 mL) was stirred at room temperature for 55 min. The TFA was then removed in vacuo, and the residue was treated with CH_2Cl_2/toluene and concentrated in vacuo to leave a white solid which dried in vacuo over P_2O_5 (0.142 g). This solid was dissolved in anhydrous DMF (1.3 mL) under argon. The reaction mixture was placed in an ice bath, and then a solution of 3-(aminomethyl)pyridine (0.028 g, 0.255 mmol) in anhydrous DMF (0.2 mL) was added followed by PyBOP (0.093 g, 0.178 mmol) and, finally, diisopropylethylamine (0.154 g, 1.19 mmol). The reaction mixture was stirred at 0 °C for 3 min; the ice bath was then removed, and stirring was continued under argon for 3 h. The clear solution was then partitioned between AcOEt (120 mL) and saturated aqueous $NaHCO_3$ (60 mL). The organic layer was washed with more saturated aqueous $NaHCO_3$ (50 mL) and brine (40 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on gradient elution with MeOH in CH_2Cl_2 (5−13%), afforded a glass. Reprecipitation from CH_2Cl_2/hexanes afforded a white solid which was collected by filtration, washed with hexanes, and dried in vacuo over P_2O_5 (0.070 g, 70%): mp 120 °C (softens); ^{1}H NMR (DMSO-d_6) 2.15 (s, 3H, *N*-Me piperazine), 2.23 (br s) and 2.49 (br s obscured, 8H, $N(CH_2CH_2)_2N$-Me), 3.17 (s, poorly resolved triplet; 1H, C≡CH), 3.60 (s, 3H, N^3-Me), 3.61 (s, 2H, 2-CH_2), 4.36 (d, J = 1.72 Hz, 2H, $CH_2C{\equiv}C$), 4.45 (d, J = 5.8 Hz, 2H, $CONHCH_2$), 4.77 (s, 2H, 6-CH_2), 6.78 (d, J = 8.9 Hz, 2H, 3,5′-ArH), 7.31 (dd, J = 4.8, 7.8 Hz, 1H, pyr 5-H), 7.68 (d, J = 7.8 Hz, pyr 4-H), 7.75 (d, J = 8.8 Hz, 2H, 2′,6′-ArH), 7.80, 7.92 (2 × s, 2H, 5-H, 8-H), 8.42 (d, J = 4.9 Hz, pyr 6-H), 8.52 (d, J = 1.6 Hz, 1H, 2-H pyr), 8.72 (t, J = 5.82 Hz, 1H, CONH); MS (FAB, *m*/*z*) 584 and 586 [(M + H)$^+$; 100 and 36%, respectively; Cl isotopic pattern]. Anal. ($C_{32}H_{34}ClN_7O_2$·1.2H_2O) C, H, N, Cl.

2-Acetoxymethyl-6-bromomethyl-7-chloro-3,4-dihydroquinazolin-4-one (24). To a suspension of **13** (2.00 g, 7.5 mmol) in anhydrous CCl_4 (120 mL) was added NBS (1.47 g, 8.3 mmol) followed by dibenzoyl peroxide (7.0 mg) under argon. The reaction mixture was placed in a preheated oil bath at 120 °C and was stirred at this temperature for 3.5 h while being illuminated. The solvent was removed in vacuo, and the residue was purified twice by column chromatography using 40% AcOEt in $CHCl_3$ as eluant (1.02 g, 40%): mp 190−195 °C; ^{1}H NMR (DMSO-d_6) 2.14 (s, 3H, CH_3CO), 4.91, 4.97 (2 × s, 4H, 2-CH_2 and 6-CH_2), 7.78 (s, 1H, 8-H), 8.36 (s, 1H, 5-H), 12.61 (s, 1H, N^3-H). This product was used in the next experiment without any further purification.

***tert*-Butyl 4-[*N*-[2-Acetoxymethyl-7-chloro-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]-benzoate (25).** To a stirred solution of **24** (1.02 g, 3.0 mmol) in anhydrous DMF (100 mL) was added *tert*-butyl 4-*N*-(prop-2-ynyl)aminobenzoate (0.78 g, 3.4 mmol) followed by 2,6-lutidine (1.23 mL, 10.6 mmol). The reaction mixture was placed in a preheated oil bath at 120 °C and stirred at this temperature for 16 h under argon; it was then allowed to cool to room temperature. The solvent was removed in vacuo, and the residue was partitioned between AcOEt (300 mL) and half-saturated brine (300 mL). The aqueous layer was extracted with more AcOEt (2 × 100 mL); the combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by column chromatography, on elution with 30% AcOEt in $CHCl_3$, afforded a white solid (0.713 g, 48%): mp 219−220 °C; ^{1}H NMR (DMSO-d_6) 1.49 (s, 9H, Bu^t), 2.12 (s, 3H, CH_3CO), 3.25 (s, 1H, C≡CH), 4.40 (s, 2H, $CH_2C{\equiv}C$), 4.78 (s, 2H, 6-CH_2), 4.94 (s, 2H, 2-CH_2), 6.78 (d, J = 8.8 Hz, 2H, 3′,5′-ArH), 7.72 (d, J = 8.8 Hz, 2H, 2′,6′-ArH), 7.81, 7.83 (2 × s, 5-H, 8-H), 12.52 (s, 1H, N^3-H); FAB-HRMS measured 495.1551, calcd for $C_{26}H_{26}N_3ClO_5$ 495.1561. Anal. ($C_{26}H_{26}ClN_3O_5$) C, H, N.

***tert*-Butyl 4-[*N*-[7-Chloro-2-hydroxymethyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]-benzoate (26).** To a solution of **25** (0.070 g, 0.14 mmol) in THF (2.7 mL) was added dropwise aqueous NaOH (1 N, 0.27 mL, 0.27 mmol) followed by H_2O (0.2 mL). The reaction mixture was stirred at room temperature for 2 h, and then the THF was removed in vacuo. The residue was suspended in H_2O (10 mL), and the pH was adjusted to ∼5 with 1 N HCl. The white precipitate was collected by filtration and dried in vacuo, and then it was reprecipitated from CH_2Cl_2/hexanes to afford the title compound **26** as a white solid (0.044 g, 70%): mp 185−187 °C; ^{1}H NMR (DMSO-d_6) 1.49 (s, 9H, Bu^t), 3.25 (s, 1H, C≡CH), 4.35, 4.38 (2 × s, 4H, $CH_2C{\equiv}C$ and 2-CH_2), 4.78 (s, 2H, 6-CH_2), 5.62 (br s, 1H, OH), 6.78 (d, J = 8.7 Hz, 2H, 3′,5′-ArH), 7.73 (d, J = 8.6 Hz, 2H, 2′,6′-ArH), 7.78, 7.84 (2 × s, 2H, 5-H, 8-H), 12.07 (s, 1H, N^3-H); FAB-HRMS measured 453.1463, calcd for $C_{24}H_{24}ClN_3O_4$ 453.1455. Anal. ($C_{24}H_{24}ClN_3O_4$·0.25H_2O) C, H, N.

***tert*-Butyl 4-[*N*-[7-Chloro-2-methanesulfonyloxymethyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (27).** To a solution of **26** (0.250 g, 0.55 mmol) in anhydrous DMF (6 mL) under argon was added methanesulfonic anhydride (0.191 g, 1.10 mmol) followed immediately by Et_3N (0.27 mL, 1.93 mmol). The clear solution was stirred at room temperature for 45 min, and then it was partitioned between AcOEt (200 mL) and saturated aqueous $NaHCO_3$ (60 mL). The organic layer was washed with more saturated aqueous $NaHCO_3$ (60 mL) and brine (60 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with 40% AcOEt in CH_2Cl_2, afforded a white solid (0.212 g, 73%): mp 178−181 °C; ^{1}H NMR (DMSO-d_6) 1.49 (s, 9H, CO_2Bu^t), 3.23 (s, 1H, C≡CH), 3.20 (s, 3H, SO_2Me), 4.39 (s, 2H, $CH_2C{\equiv}C$), 4.78 (s, 2H, 6-CH_2), 5.11 (s, 2H, 2-CH_2), 6.78 (d, J = 9.0 Hz, 2H, 3′,5′-ArH), 7.72 (d, J = 8.8 Hz, 2H, 2′,6′-ArH), 7.85, 7.88 (2 × s, 2H, 5-H, 8-H); MS (ESI, *m*/*z*) 554 and 556 [(M + Na)$^+$; 100 and 38%, respectively; Cl isotopic pattern]. Anal. ($C_{25}H_{26}ClN_3O_6S$) C, H, N.

***tert*-Butyl 4-[*N*-[7-Chloro-2-(piperidin-1-yl)methyl-4-oxo-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)-amino]benzoate (28).** To a solution of **27** (0.201 g, 0.38 mmol) in anhydrous DMF (5 mL) was added piperidine (0.323 g, 3.8 mmol), and the clear solution was stirred at room temperature for 2.5 h. The reaction mixture was then partitioned between AcOEt (200 mL) and 5% aqueous Na_2CO_3 (70 mL). The organic layer was washed with 5% aqueous Na_2CO_3 (70 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with AcOEt/CH_2Cl_2 (v/v, 1/1), afforded a white solid (0.179 g, 91%): mp 208−210 °C; ^{1}H NMR (DMSO-d_6) 1.36 (m), 1.48 (m, obscured), 1.49 (s, 15H, Bu^t and piperidine $CH_2CH_2CH_2$), 2.42, (br s, 4H, piperidine CH_2NCH_2), 3.61 (s, 2H, 2-CH_2), 3.22 (s, 1H, C≡CH), 4.38 (s, 2H, $CH_2C{\equiv}C$), 4.77 (s, 2H, 6-CH_2), 6.77 (d, J = 9.0 Hz, 2H, 3′,5′-ArH), 7.72 (d, J = 8.9 Hz, 2′,6′-ArH), 7.79, 7.83 (2 × s, 2H, 5-H, 8-H), 11.96 (s, 1H, N^3-H); MS (ESI, *m*/*z*) 521 and 523 [(M + H)$^+$; 100 and 35%, respectively; Cl isotopic pattern]. Anal. ($C_{29}H_{33}ClN_4O_3$) C, H, N, Cl.

***tert*-Butyl 4-[*N*-[7-Chloro-3-methoxycarbonylmethyl-4-oxo-2-piperidin-1-ylmethyl-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (29a).** To a solution of **28** (0.096 g, 0.18 mmol) in anhydrous DMF (5 mL) under argon was added NaH (60% dispersion in mineral oil, 8 mg, 0.2 mmol) in one portion. The reaction mixture was stirred at room temperature for 3 min, and then methyl bromoacetate (0.141 g, 0.9 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 24 h, and then partitioned between AcOEt (150 mL) and half-saturated brine (100 mL). The organic layer was washed with more brine (100 mL). The combined aqueous washings were extracted with AcOEt (2 × 50 mL). The combined AcOEt extracts were washed with brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with $CHCl_3$, afforded a white solid (0.071 g, 67%): mp 148−150 °C; ^{1}H NMR (DMSO-d_6) 1.36 (m, 6H, piperidine $CH_2CH_2CH_2$), 1.50 (s, 9H, Bu^t), 2.32 (m, 4H, piperidine CH_2NCH_2), 3.23 (s, 1H, C≡CH), 3.58 (s, 2H, 2-CH_2), 3.67 (s, 3H, CO_2Me), 4.40 (d, J = 2.0 Hz, 2H, $CH_2C{\equiv}C$), 4.80 (s, 2H, 6-CH_2), 4.87 (s, 2H, N^3-CH_2), 6.77 (d, J = 9.0 Hz, 2H, 3′,5′-ArH), 7.72 (d, J = 8.9 Hz, 2′,6′-ArH), 7.86, 7.87 (2 × s, 2H, 5-H, 8-H); MS (FAB, *m*/*z*) 593 and 595

[(M + H)$^+$; 100 and 36%, respectively; Cl isotopic pattern]. FAB-HRMS measured 593.2506, calcd for $C_{32}H_{38}ClN_4O_5$ 593.2531.

***tert*-Butyl 4-[*N*-[7-Chloro-3-diethylcarbamoylmethyl-4-oxo-2-(piperidin-1-yl)methyl-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (29b).** To a solution of **28** (0.088 g, 0.17 mmol) in anhydrous DMF (2 mL) was added NaH (60% dispersion in mineral oil, 8.2 mg, 0.2 mmol) in one portion. The reaction mixture was stirred at room temperature for 3 min under argon, and then a solution of *N*,*N*-diethylbromoacetamide[8] in anhydrous DMF (0.4 mL) was added. The clear solution was stirred at room temperature for 2.5 h, and then partitioned between AcOEt (40 mL) and brine (40 mL). The aqueous layer was extracted with more AcOEt (2 × 30 mL), and the combined AcOEt extracts were washed with brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with a gradient of AcOEt in hexane (20 to 50%), afforded a white solid (0.081 g, 76%): mp 95−97 °C; ^{1}H NMR ($CDCl_3$) 1.13, 1.33 (2 × t, J = 7.1 Hz, 6H, 2 × CH_2CH_3), 1.42 (m, obscured; 6H, piperidine $CH_2CH_2CH_2$), 1.55 (s, 9H, Bu^t), 2.27 (s, 1H, C≡CH), 2.42 (m, 4H, piperidine CH_2NCH_2), 3.41 (m, 4H, 2 × CH_2CH_3), 3.52 (s, 2H, 2-CH_2), 4.17 (s, 2H, CH_2C≡C), 4.75 (s, 2H, 6-CH_2), 5.30 (s, 2H, N^3-CH_2), 6.73 (d, J = 9.0 Hz, 2H, 3′,5′-ArH), 7.78, 8.08 (2 × s, 2H, 5-H, 8-H), 7.86 (d, J = 9.02 Hz, 2H, 2′,6′-ArH); MS (ESI, *m*/*z*) 634 and 636 [(M + H)$^+$; 100 and 37%, respectively; Cl isotopic pattern].

***tert*-Butyl 4-[*N*-[7-Chloro-4-oxo-2-(piperidin-1-yl)methyl-3-piperidinocarbonylmethyl-3,4-dihydroquinazolin-6-ylmethyl]-*N*-(prop-2-ynyl)amino]benzoate (29c).** To a solution of **28** (0.052 g, 0.10 mmol) in anhydrous DMF (1 mL) was added NaH (60% dispersion in mineral oil, 5.00 mg, 0.12 mmol) in one portion. The reaction mixture was stirred at room temperature for 3 min under argon, and then a solution of 1-(bromoacetyl)piperidine[9] in anhydrous DMF (0.2 mL) was added. The clear solution was stirred at room temperature for 3 h, and then partitioned between AcOEt (40 mL) and brine (40 mL). The aqueous layer was extracted with AcOEt (2 × 20 mL), and the combined AcOEt extracts were washed with brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification by column chromatography, on elution with a gradient of AcOEt in hexanes (40−50%), afforded a white solid (0.040 g, 62%): mp >104 °C; ^{1}H NMR (DMSO-d_6) 1.30−1.65 (m, 12H), 2.36 (br s, 4H) and 3.40 (m, 4H) (piperidine protons), 1.49 (s, 9H, Bu^t), 3.21 (s, 1H, C≡CH), 3.47 (s, 2H, 2-CH_2), 4.39 (s, 2H, CH_2C≡C), 4.80 (s, 2H, 6-CH_2), 5.10 (s, 2H, N^3-CH_2), 6.78 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.83, 7.84 (2 × s, 2H, 5-H, 8-H), 7.73 (d, J = 8.8 Hz, 2H, 2′,6′-ArH); MS (ESI, *m*/*z*) 646 and 648 [(M + H)$^+$; 100 and 36%, respectively; Cl isotopic pattern]. Anal. ($C_{36}H_{44}ClN_5O_4$·0.5H_2O) C, H, N.

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Supporting Information Available: Complete experimental procedures for the preparation of compounds **4b**−**j**, **5b**−**j**, **31a**−**c**, **32**, and **33**. This material is available free of charge via the Internet at http//pubs.acs.org.

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