Quinazoline Antifolate Thymidylate Synthase Inhibitors: Replacement of Glutamic Acid in the C2-Methyl Series

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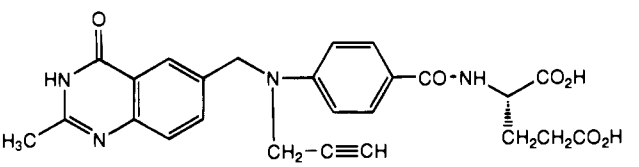
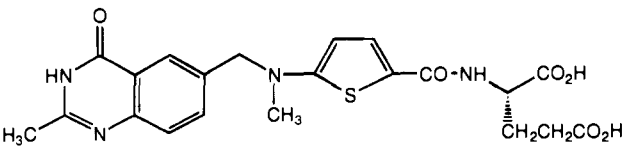
The synthesis of a series of analogues of the potent thymidylate synthase (TS) inhibitor *N*-[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (ICI 198583, **1**) is described in which the glutamic acid residue has been replaced by other α-amino acids. Most of these analogues were prepared by coupling of *tert*-butyl-4-(prop-2-ynylamino)benzoate (**37**) with 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline (**34**) followed by deprotection of the *tert*-butyl ester to the acid and azide-mediated coupling to the appropriate amino acid or amino acid ester. In cases where the amino acid ester was unreactive with the acid azide, a modification was used in which the quinazolinone moiety was protected as its 3-(pivaloyloxy)methyl derivative. This permitted the generation of the more reactive acid chloride of the *p*-aminobenzoate unit. In general these modifications result in compounds that have equivalent potency to **1** as inhibitors of isolated TS except where the amino acid lacks a lipophilic α-substituent. These compounds appear to require the reduced folate carrier (RFC) for transport into cells, but since they are not converted intracellularly into polyglutamated forms, they have a lower level of cytotoxicity compared to 1. The removal of the α-carboxylic acid has given a second set of analogues of **1** which contain simple alkyl amide, benzyl, substituted benzyl, and heterocyclic benzyl amide derivatives. These are considerably less potent than **1** as TS inhibitors but display 1-10 μM cytotoxicities due to the fact that they do not require RFC transport and can presumably readily enter cells by passive diffusion through the cell membrane. Molecular modeling and NMR studies indicated that the incorporation of, respectively, 7-methyl and 2′-fluoro substituents would favor the optimum conformation of these molecules for interaction with the TS enzyme. Accordingly, these substituents were incorporated into selected examples to give the series of analogues **47-55**. These all show enhanced (~10-fold) inhibition of TS compared to their unsubstituted counterparts. In the substituted benzylamides (**51**, **52**) and heterocyclic benzyl amides (**53-55**) the ability to enter cells by passive diffusion results in highly potent (<1 μM) cytotoxic agents.

The principle of effective antitumour chemotherapy with a specific inhibitor of thymidylate synthase (TS) was established in clinical trials[1-3] of the quinazoline-based antifolate CB3717 (*N*10-propargyl-5,8-dideazafolic acid). The unacceptable renal toxicity that caused this compound to be withdrawn from the clinic results from its poor aqueous solubility.[4,5] A search for more soluble analogues of CB3717 resulted in the synthesis of several series of 2-methylquinazoline analogues[6-9] that are considerably more potent than CB3717 as cytotoxic agents and are devoid of renal toxicity at therapeutic doses. Detailed studies of the biochemical pharmacology of two of these 2-methylquinazolinone analogues, ICI 198583 (**1**)[10] and Zeneca ZD1694 (Tomudex, **2**)[11,12] have demonstrated that their enhanced cytotoxicities result from rapid intracellular localization via the reduced folate carrier (RFC) and then extensive metabolism by folylpolyglutamate synthetase (FPGS) to polyglutamated derivatives which are well-retained within cells by virtue of their increased polyanionic character and are up to 100 times more potent than the parent drugs as inhibitors of TS. Such active transport



1 ICI 198583



2 ZD1694 (Tomudex)

of classical (i.e., containing the glutamic acid residue) antifolates into cells and their intracellular polyglutamation are processes critical to their cytotoxicity[13] but can ultimately lead to mechanisms of resistance.[14] The latter compound, Tomudex, has recently completed phase I and II clinical trials[15-17] and is currently in phase III trials for colorectal cancer. The fact that tumor cells can acquire resistance to folate-based antimetabolites by deletion or modification of either the

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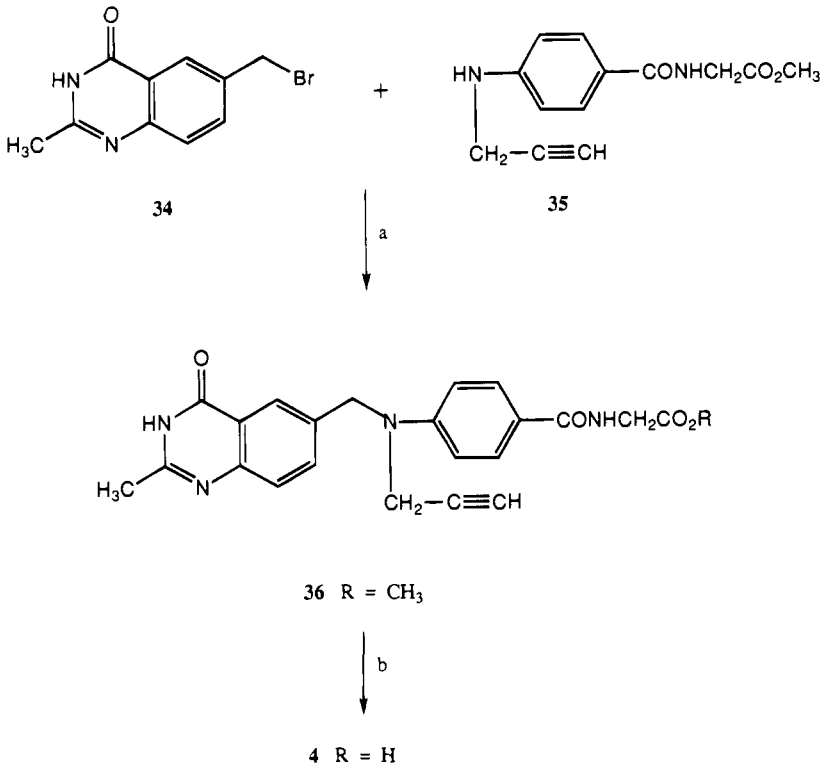
Scheme 1[a]



[a] (a) $CaCO_3$, DMF, 25 °C (method A); (b) 1 N NaOH, EtOH then 2 N HCl (method B).

RFC[18] or FPGS[19] has stimulated the search for compounds that do not require these mechanisms to express potent antitumor activity. Modifications to the γ-position of the glutamic acid residue in the dihydrofolate reductase inhibitor, methotrexate (MTX), have resulted in compounds that are poor substrates or nonsubstrates for FPGS. Such compounds, exemplified by the γ-fluoro[20] derivative, still require active transport by the RFC to retain the potent cytotoxicity of the parent. Recent approaches to the design of lipophilic TS inhibitors[21-23] have produced cytotoxic agents that require neither active transport by the RFC nor intracellular polyglutamation for their activity. One such analogue, 2-amino-3,4-dihydro-6-methyl-4-oxo-5-(4-pyridylthio)quinazoline (AG 337)[23] has demonstrated antitumor activity in animal models and has entered a phase I clinical study.[24]

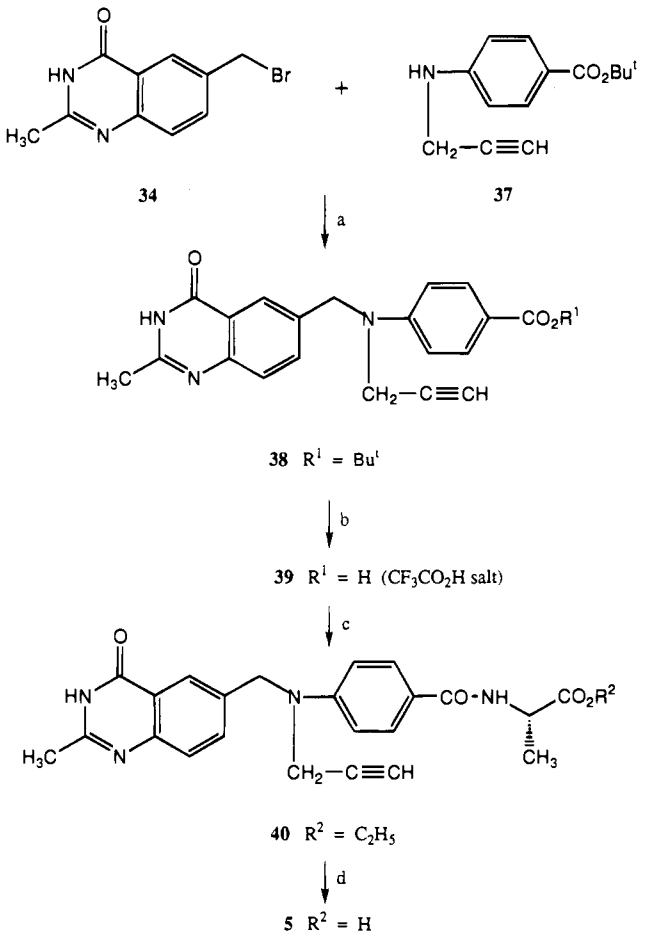
The TS inhibitory and cytotoxic potencies of **1** suggested that this compound should be a good starting point for molecular modification in the search for antifolate TS inhibitors that do not require active transport into cells or polyglutamation to give potent antitumor activity. This paper describes the synthesis and biological activities of analogues of **1** that were prepared to explore this hypothesis. The initial strategy was to replace the glutamic acid moiety with α-amino acids that could not be substrates for FPGS since they lack the γ-carboxylic acid. Subsequently analogues containing neither the α- nor the γ-carboxylic acids were prepared in order to explore the possibility of identifying agents that could diffuse into cells and therefore not require RFC uptake.

Chemistry

The general methods for the synthesis of these new non-glutamate analogues of **1** are outlined for typical examples in Schemes 1-4. The overall strategy involved the coupling of the (bromomethyl)quinazolinone **34**[6] with an esterified amino acid derivative of 4-(*N*-prop-2-ynylamino)benzoic acid (as in **35**, Scheme 1) or with *tert*-butyl ester (**37**, Scheme 2). In the latter sequence the *tert*-butyl ester **38** was deprotected (CF_3CO_2H) to the carboxylic acid **39** which was activated by diphenyl phosphorazidate (DPPA) to the azide **41** (Scheme 3). This azide could be prepared in situ and condensed with the amine moiety in the presence of triethylamine (methods C and E). Alternatively, **41** could be isolated as an analytically pure solid and then condensed with an amino acid in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (method D). A number of amines failed to condense with **41**. For these examples the carboxylic acid was activated as the acid chloride **45** although this method required protection of the quinazolinone 3-NH as the (pivaloyloxy)methyl (POM) derivative (method G, Scheme 4).

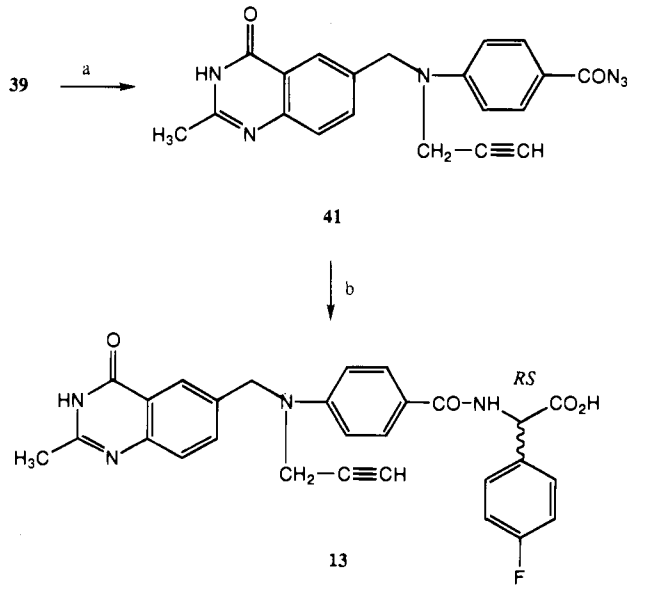
The synthesis of the examples **47-55** which incorporated a C7-methyl substituent in the quinazolinone and a 2′-fluoro substituent in the *p*-aminobenzoate (PABA) unit required the preparation of the key carboxylic acid **62** which again utilized POM protection of the quinazolinone NH. The quinazolinone precursor **58** was prepared (Scheme 5) from the known anthranilic acid derivative **56**[25] via cyclization with acetic anhydride followed by treatment with aqueous ammonia. Incorporation of the POM protecting group was followed by bromination with *N*-bromosuccinimide (NBS) in refluxing CCl_4. Under these conditions the 6-bromomethyl compound **60** was the only monobrominated product formed, although this required purification by chromatography to remove small amounts of several dibrominated derivatives. The regiospecificity of the bromination of **59** to **60** was confirmed in an NOE experiment

Scheme 2[a]



[a] (a) $CaCO_3$, DMF, 25 °C; (b) CF_3CO_2H, 25 °C; (c) DPPA, Et_3N, L-alanine ethyl ester (method C); (d) 1 N NaOH, EtOH, then 2 N HCl.

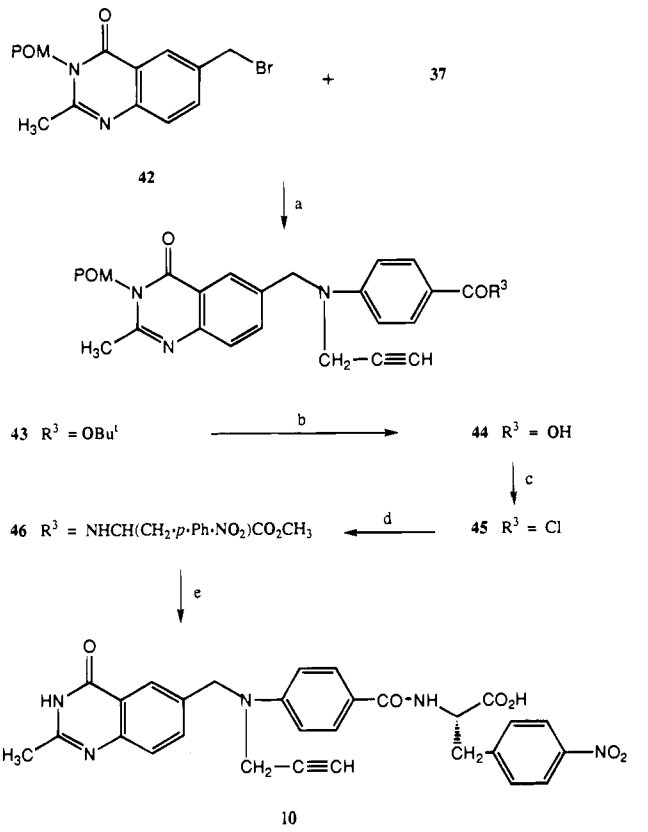
Scheme 3[a]



[a] (a) DPPA, Et_3N, DMF; (b) D,L-2-(4-fluorophenyl)glycine, DBU, DMF (method D).

in which irradiation of the CH_2Br signal at δ 4.59 gave enhancements in the intensities of the signals at δ 8.19 (quinazoline 5-H) and δ 2.56 (7-CH_3). The fluorinated PABA unit **67** was prepared as shown in Scheme 6 by our earlier method,[7] adapted to give the *tert*-butyl ester.

Scheme 4[a]



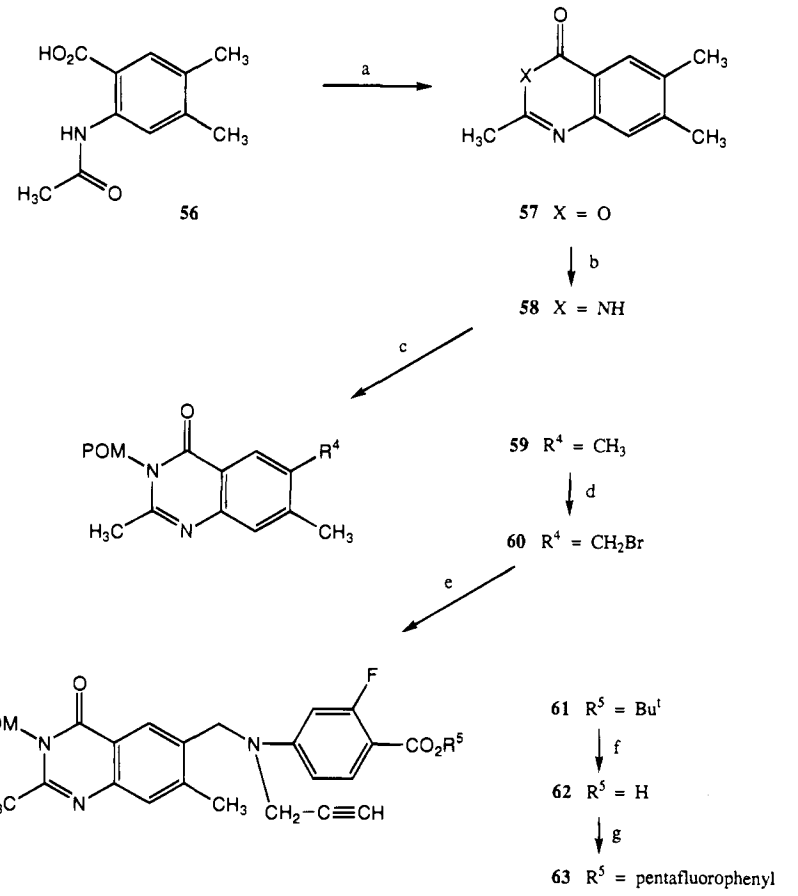
[a] (a) 2,6-lutidine, DMF, 55 °C; (b) CF_3CO_2H; (c) oxalyl chloride, DMF (cat.), CH_2Cl_2 (method G); (d) L-(4-nitrophenyl)alanine methyl ester; (e) 1 N aqueous NaOH, EtOH then 2 N HCl.

Coupling of **60** and **67** in the presence of 2,6-lutidine gave the *tert*-butyl ester **61** which was converted to the free acid **62** by a brief treatment with CF_3CO_2H. Amide formation was achieved using either the acid chloride route (method G) or conversion of the acid **62** to the pentafluorophenyl ester **63**. This active ester could be isolated as a stable crystalline solid which underwent coupling under mild conditions (method H) with a range of amines (Scheme 7). A simple saponification using 1 N aqueous NaOH simultaneously removed the POM protecting group and, where present, the alkyl group of the amino acid ester. Alternatively when only POM group removal was required, this final deprotection step could be accomplished by exposure to a saturated solution of ammonia in MeOH (method I, Scheme 8).

Biological Evaluation

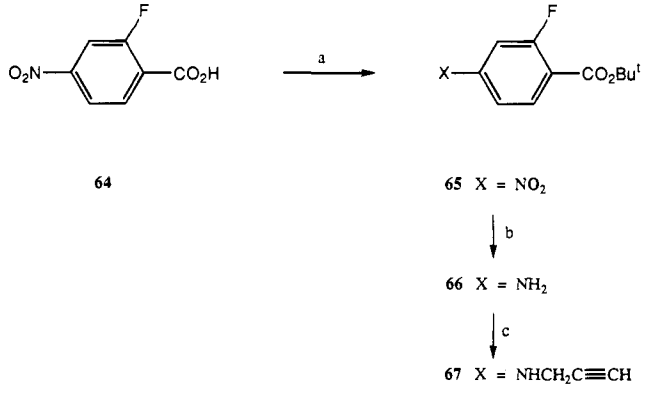
The antifolate diacids listed in Tables 1 and 2 were tested as inhibitors of TS partially purified from L1210 mouse leukemia cells that overproduce TS.[26] The partial purification and assay method used was as previously described and used a (±)-5,10-methylenetetrahydrofolate concentration of 200 μM.[26] The results are expressed as IC_{50} values, that is the concentration of compound that will inhibit the control reaction rate by 50%. The compounds were also tested for their inhibition of the growth of L1210 cells in culture, and the results are expressed as the concentration of compound required to inhibit cell growth by 50% (IC_{50}). The L1210:1565 cell line[27] has acquired resistance to the antitumor antibiotic CI-920.[28] Evidence suggests that this agent enters cells via the reduced folate mechanism and that the L1210:1565 line is resistant due to a very

Scheme 5[a]



[a] (a) Ac_2O, xylene, reflux; (b) 35% aqueous NH_4OH, 2 N NaOH, 60 °C; (c) NaH, chloromethyl pivalate, DMF; (d) *N*-bromosuccinimide, AIBN, CCl_4, reflux; (e) **67**, 2,6-lutidine, DMF, 95 °C; (f) CF_3CO_2H; (g) pentafluorophenol, DCCI, 4-pyrrolidinopyridine, Et_3N, DMF.

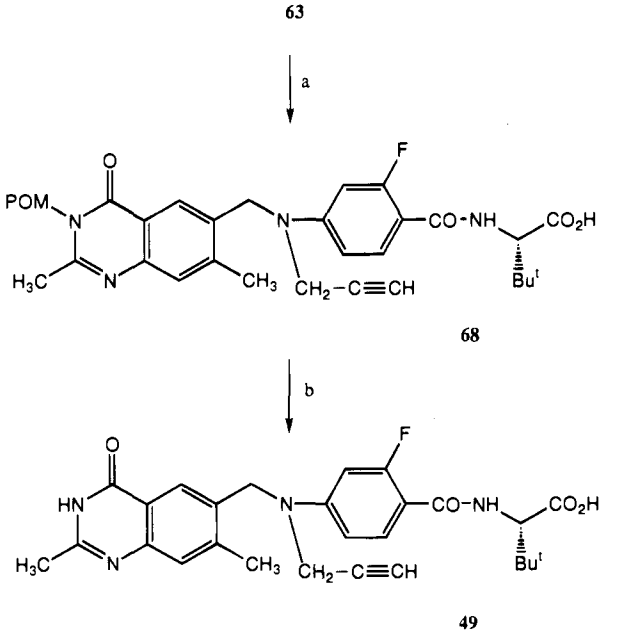
Scheme 6[a]



[a] (a) *p*-$CH_3C_6H_4SO_2Cl$, Bu^tOH, pyridine; (b) H_2, 10% Pd/C, EtOAc; (c) propargyl bromide, 2,6-lutidine, DMF, 95 °C.

much reduced drug uptake and hence it is cross resistant to MTX (~200-fold).[29] Both cell lines were grown by suspension culture in RPMI medium without sodium bicarbonate but containing 20 mM HEPES[30] and supplemented with 10% horse serum (L1210) or 10% fetal calf serum (L1210:1565). Incubation times for the 5 mL cultures were 48 (L1210) and 72 h (L1210:1565). The initial cell concentration was 5×10^4 mL^{-1}. For the thymidine protection experiments, the L1210 cells were co-incubated with the compounds at concentrations of 10 times the IC_{50} values and 10 μM thymidine. All cell counts were performed with a Model ZM Coulter counter. The cell doubling times were 12 (L1210) and 24 h (L1210:1565).

Scheme 7[a]

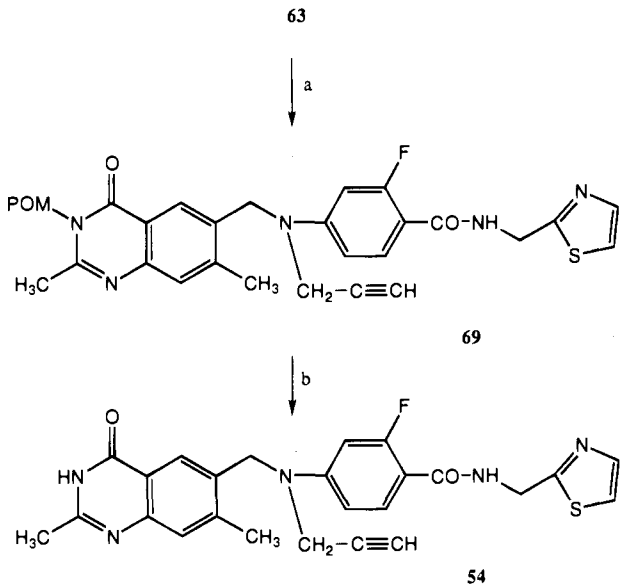


[a] (a) L-*tert*-Leucine, Et_3N, 1-hydroxybenzotriazole, DMA (method H); (b) 1 N aqueous NaOH, EtOH then 2 N aqueous HCl.

Results and Discussion

The replacement of the glutamic acid moiety in **1** with a range of α-amino acids, to give compounds **3**–**14**, in general resulted in retention of potency against the isolated TS enzyme. The one exception was the glycine

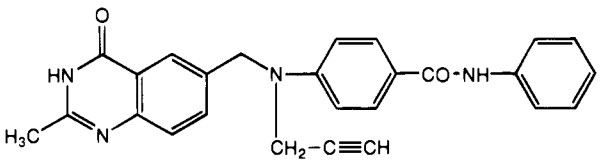
Scheme 8[a]



[a] (a) 2-Aminomethylthiazole dihydrochloride, Et_3N, 1-hydroxybenzotriazole, DMA; (b) NH_3, MeOH (method I).

analogue **4** which was more than 10-fold less potent, suggesting the presence of a hydrophobic binding pocket in the region normally occupied by the glutamate side chain. These results parallel the pattern seen with the glycine and L-alanine isosteres of CB3717.[31] This pocket will accept a wide variety of groups from small alkyl through branched alkyl to substituted phenyl and benzyl rings. On the other hand, the growth inhibitory potencies of these analogues are considerably reduced compared to **1**, which presumably reflects their inability to act as substrates for FPGS. The high relative resistance values of the alkyl side chain amino acids in the L1210:1565 cell line indicate that these compounds probably enter cells by the RFC.[29] On the other hand, when the relative resistance was determined for two examples **11** and **14** of aryl side chains, then the low values of 2 and 1.4, respectively, suggest that these compounds are not internalised via the RFC. The fact that these compounds do give growth inhibition, albeit at a lower level of potency, indicates a low level of entry, possibly by passive diffusion by virtue of their solubility in the lipid bilayer of the cell membrane.

Removal of the α-carboxylic acid of the alkyl amino acid analogues yielded the alkyl amides **16** and **17** which are ~50-fold worse than the corresponding α-acids as inhibitors of TS but which have equivalent growth inhibitory potencies, presumably due to an enhanced ability to enter cells by diffusion. In the aromatic amino acids, this change gave a series of benzyl amides **18**–**24**. These have equivalent TS inhibitory potency to the alkyl amides but with enhanced growth inhibition. Introduction of polarity into the benzyl amides had a variable response, e.g., chlorine is detrimental whereas nitro is beneficial. Similar results were obtained with a variety of heterocyclic benzyl amides (**25**–**32**). The consistently low values for the L1210:1565 relative resistance of the non-acid-containing compounds supports non-RFC cell entry. The log *P* (octanol/water) values have been measured for representative examples of these non-acids. Respective values of 2.05, 3.4, and 3.7 for **16**, **18**, and **21** show that the compounds are in the lipophilicity range where diffusion through the cell membrane is the predominant mode of entry in the absence of any carrier mechanism.[32] The simple anilide substitution (**33**) retains TS potency although cytotoxicity is diminished. Thymidine protection studies on representative examples confirmed that TS is the predominant cytotoxic locus for these series of compounds.



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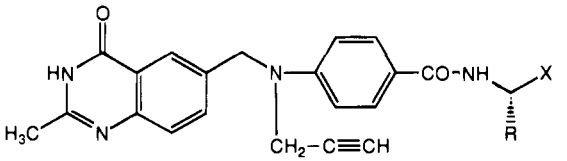
Molecular modeling studies[33,34] based on the crystal structures[35,36] of ternary complexes of CB 3717, a pyrimidine nucleotide and *E. coli* TS indicated that a 7-methyl substituent would reinforce the partially folded conformation with the *p*-aminobenzoate (PABA) ring inclined at an angle of 65° to the quinazolinone, which is optimum for binding to the enzyme. Our earlier studies[7,8] had also shown that a 2′-fluoro substituent gives a 2–3-fold enhancement of TS inhibition and cytotoxicity in the PABA–glutamate series, presumably through the stabilization of the almost planar conformation in this region of the molecule due to a hydrogen bond between the fluorine atom and the amidic NH of the glutamate (revealed by NMR studies). This conformation allows the hydrogen bonding through water of the PABA carbonyl group to a chain carbonyl of the C-terminus of the enzyme,[35,36] an effect that would also be enhanced by the electron-withdrawing properties of the fluorine atom. The incorporation of these two substituents into the ICI 198583 molecule, to give **47**, did indeed give an enhancement in TS inhibition (5-fold). However, in this glutamic acid series, examples **1** and **47** have similar potencies as inhibitors of cell growth. This is due to the fact that C7-methyl-substituted antifolates are not substrates for FPGS,[37] and therefore **47** does not form intracellular polyglutamates. The observed cytotoxicity of **47** is therefore entirely due to the activity of the parent monoglutamate. Accordingly, the 7-methyl-2′-fluoro derivatives **48**–**55** of some of the more potent non-glutamate-containing analogues were prepared. These derivatives again showed a consistent improvement in TS inhibitory potency over their unsubstituted parents. In the "lipophilic" derivatives **51**–**55** this translated into a consistent (6–10-fold) enhancement of growth inhibitory potency which again supports the view that these compounds do not require the RFC to enter cells. This conclusion is also supported by relative resistance values for these compounds of ≤1 in the L1210:1565 line. In the case of the α-acid analogues **48**–**50**, the situation is somewhat more complex since the L1210:1565 relative resistance values suggest that these compounds utilize RFC-mediated uptake to varying degrees. In the *m*-nitrophenyl case, the parent **14** appears to enter cells mainly by passive diffusion and hence the substituted derivative **50** shows enhanced growth inhibition. On the other hand, the branched alkyl (valine and *tert*-leucine) analogues **7**, **8**, **48**, and **49** appear to be transported by the RFC but poorly

Table 1. Preparation and in Vitro Activity of Quinazoline Antifolates **3**–**33**



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compd	X	R	formula[a]	method	yield (%)	mp (°C)	inhibn of TS: IC_{50} (μM)	inhibition of cell growth in culture: IC_{50} (μM) L1210	protection at 10IC_{50} (% of control)	L1210:1565	L1210:1565 relative resistance
1[b]	CO_2H	$CH_2CH_2CO_2H$	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$				0.040	0.09		8.2	96
3	CO_2H	$CH_2CH_2CONH_2$	$C_{25}H_{25}N_5O_5{\cdot}1.5H_2O$	E, F	30	155–157	0.067	2.2	92	40	18
4	CO_2H	H	$C_{22}H_{20}N_4O_4{\cdot}1.5H_2O$	A, B	48	240–250[c]	0.52	>100			
5	CO_2H	CH_3	$C_{23}H_{22}N_4O_4{\cdot}1.25H_2O$	C	82	165–170	0.15	25	88		
6	CO_2H	$CH_2CH_2CH_3$	$C_{25}H_{26}N_4O_4{\cdot}H_2O$	D	64	138–141	0.042	2.5	91	34	14
7	CO_2H	$CH(CH_3)_2$	$C_{25}H_{26}N_4O_4{\cdot}0.75H_2O$	C	91	210–211	0.058	0.96	100	23	23
8	CO_2H	$C(CH_3)_3$	$C_{26}H_{28}N_4O_4{\cdot}1.5H_2O$	D	41	155–158	0.058	2	100	14	7
9	CO_2H	CH_2Ph	$C_{29}H_{26}N_4O_4{\cdot}H_2O$	A, B	69	152–155	0.17	41			
10	CO_2H	CH_2Ph-*p*-NO_2	$C_{29}H_{25}N_5O_6{\cdot}1.75H_2O$	D	80	234–237	0.042	>20			
11	CO_2H	phenyl	$C_{28}H_{24}N_4O_4{\cdot}1.25H_2O$	D	36	158–160	0.034	15		30	2
12	CO_2H	Ph-*m*-OH	$C_{28}H_{24}N_4O_5{\cdot}2Me_2CO$	D	18	164–167	0.16	90			
13	CO_2H	Ph-*p*-F (RS)	$C_{28}H_{23}FN_4O_4{\cdot}1.75H_2O$	D	62	197–202	0.089	74			
14	CO_2H	Ph-*m*-NO_2(RS)	$C_{28}H_{23}N_5O_6{\cdot}2H_2O$	G	75	173–175	0.024	9		13	1.4
15	H	H	$C_{21}H_{20}N_4O_2{\cdot}2H_2O$	E	86	235–240	4.20	25			
16	H	CH_3	$C_{22}H_{22}N_4O_2{\cdot}0.5H_2O$	E	29	245–249	22.9	7.2			
17	H	CH_2CH_3	$C_{23}H_{24}N_4O_2{\cdot}0.66H_2O$	E	62	235–236[c]	2.26	8.6			
18	H	phenyl	$C_{27}H_{24}N_4O_2{\cdot}0.67H_2O$	E	45	231–234	0.72	4	91		
19	H	Ph-*o*-NO_2	$C_{27}H_{23}N_5O_4{\cdot}2H_2O$	E	10	225–227	0.52	1.8	96	7.5	4.2
20	H	Ph-*m*-NO_2	$C_{27}H_{23}N_5O_4{\cdot}1.12H_2O$	E	62	240–243	0.11	1.2	60	0.4	0.37
21	H	Ph-*p*-NO_2	$C_{27}H_{23}N_5O_4{\cdot}2.5H_2O$	E	46	229–233	0.32	2.2	95		
22	H	Ph-*m*-CN	$C_{28}H_{23}N_5O_2{\cdot}0.75H_2O$	D[d]	27	245–246	0.28	1.5	90	0.5	0.33
23	H	Ph-*p*-Cl	$C_{27}H_{23}ClN_4O_2{\cdot}0.75H_2O$	E	70	226–230	7.70	70			
24	H	Ph-*m*-CO_2H	$C_{28}H_{24}N_4O_4{\cdot}2.75H_2O$	E, B	42	164–168	0.27	85			
25	H	2-pyridyl	$C_{26}H_{23}N_5O_2{\cdot}H_2O$	E	41	213–218[c]	0.45	3.9	57	5	1.28
26	H	2-furyl	$C_{25}H_{22}N_4O_3{\cdot}H_2O$	D[d]	68	196–210	1.42	6			
27	H	3-furyl	$C_{25}H_{22}N_4O_3$	D[d]	40	226–227	1.38	5.5			
28	H	3-thienyl	$C_{25}H_{22}N_4O_2S{\cdot}0.75H_2O$	D[d]	38	238–245	1.46	5.5	20		
29	H	2-thiazolyl	$C_{24}H_{21}N_5O_2S{\cdot}0.6H_2O$	D[d]	21	242–243	0.76	6.	92	5	0.83
30	H	4-thiazolyl	$C_{24}H_{21}N_5O_2S{\cdot}0.6H_2O$	D[d]	18	199–202	1.72	8.	78	4.8	0.60
31	H	5-thiazolyl	$C_{24}H_{21}N_5O_2S{\cdot}0.75H_2O$	D[d]	14	242–248	0.86	1.1	93	0.4	0.36
32	H	2-thiazolyl-4-CH_3	$C_{25}H_{23}N_5O_2S{\cdot}0.75H_2O$	D[d]	34	246–252	0.77	1.7	70	1.6	0.91
33	Ph-*p*-CONHPh analogue[e]		$C_{26}H_{22}N_4O_2{\cdot}2.25H_2O$	G[f]	79	270–272	1.82	11.			

[a] Anal. C, H, N. [b] Reference 6. [c] Decomposition. [d] Method D, first stage only. [e] See structure **33**. [f] Method G, first stage only.

compared to the glutamic acid containing compounds. In the case of the valine-containing compounds **7** and **48**, the relative resistance values suggest that 7-methyl-2′-fluoro substitution causes a significant loss of internalization by the RFC, resulting in a drop in growth inhibitory potency despite an improvement in TS inhibition. The reasons for this apparent loss of RFC internalization by 7-methyl-2′-fluoro substitution have not been confirmed, but it is reasonable to suppose that conformational changes that optimize binding to TS may be detrimental for transport by the RFC. These conclusions are in accord with the affinities of representative examples of these compounds for L1210 RFC, determined by a [^{3}H]MTX competition assay.[38]

Conclusion

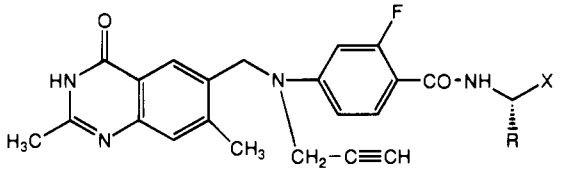
Analogues of ICI 198583 (**1**) have been identified which are potent inhibitors of TS and which show significant inhibition of cell growth. Structural changes prevent them from acting as substrates for FPGS, and depending on the nature of the modification, clear differences in affinity for the RFC have been shown. The introduction of 7-methyl and 2′-fluoro substituents into these molecules gives rise to improved binding to TS, which is reflected in enhancements of both TS and cell growth inhibition compared to the parent molecule. Thus we have identified potent cytotoxic agents that are neither actively transported by the RFC nor metabolized within cells to polyglutamated forms. These agents could substantially expand the range of tumors treated with antifolate inhibitors of TS.

Experimental Section

The general procedures used were described in the earlier paper[6] in this series.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-prop-2-ynylamino]benzoyl]glycine Methyl Ester (36). Method A.** A mixture of *N*-[4-(prop-2-ynylamino)-benzoyl]glycine methyl ester[31] (1.20 g, 4.88 mmol), the bromomethyl compound **34**[6] (1.24 g, 4.9 mmol), and $CaCO_3$ (500 mg, 4.9 mmol) in DMF (12 mL) was stirred for 72 h. The reaction mixture was filtered and evaporated, and the residue was purified by chromatography using 9:1 v/v EtOAc/MeOH as eluent to yield the methyl ester (180 mg, 9%) as a gum. The HCl salt, derived by treatment with an excess of a solution of $SOCl_2$ in MeOH, was also a gum: NMR (HCl salt; Me_2SO-d_6) δ 2.70 (s, 3 H, CH_3), 3.25 (t, 1 H, C≡CH), 3.65 (s, 3 H, OCH_3), 3.97 (d, 2 H, $CONHCH_2$), 4.40 (d, 2 H, $CH_2C{\equiv}C$), 4.89

Table 2. Preparation and in Vivo Activity of 7-Methyl-2′-fluoroquinazoline Antifolates **47–55**



47-55

compd	X	R	formula[a]	method	yield (%)	mp (°C)	inhibn of TS: IC_{50} (μM)	inhibition of cell growth in culture: IC_{50} (μM) L1210	protection at 10IC_{50} (% of control)	L1210:1565	L1210:1565 relative resistance
47	CO_2H	$CH_2CH_2CO_2H$	$C_{26}H_{25}FN_4O_6 \cdot 1.7H_2O$	H,[b] G[d]	80	248–250	0.009	0.08		5.92	74.0
48	CO_2H	$CH(CH_3)_2$	$C_{26}H_{27}FN_4O_4 \cdot 0.5H_2O$	G[c]	66	264–266	0.006	1.7	90	2.91	1.71
49	CO_2H	$C(CH_3)_3$	$C_{27}H_{29}FN_4O_4$	H, G[d]	79	258–260	0.007	0.88	98	5.28	6
50	CO_2H	Ph-*m*-NO_2(RS)	$C_{29}H_{24}FN_5O_6 \cdot 0.75H_2O$	G	77	238–239	0.0044	2.45	85	1.25	0.51
51	H	Ph-*m*-NO_2	$C_{28}H_{24}FN_5O_4 \cdot 0.75H_2O$	H, G[d]	86	256–258	0.044	0.21	66	0.18	0.86
52	H	Ph-*m*-CN	$C_{29}H_{24}FN_5O_2 \cdot H_2O$	G	69	170–175	0.048	0.23	82	0.09	0.38
53	H	2-pyridyl	$C_{27}H_{24}FN_5O_2 \cdot H_2O \cdot 0.5NaCl$	H, G[d]	57	230–233	0.114	0.62	85	0.52	0.84
54	H	2-thiazolyl	$C_{25}H_{22}FN_5O_2S \cdot H_2O$	H, I	78	270–271	0.10	0.66	80	0.50	0.76
55	H	5-thiazolyl	$C_{25}H_{22}FN_5O_2S$	H, I	57	260–262	0.11	0.62	72	0.24	0.39

[a] Anal. C, H, N. [b] Glutamic acid diethyl ester hydrochloride used as starting material. [c] Valine ethyl ester used as starting material. [d] Method G, second stage only.

(br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, 3′-H and 5′-H), 7.76 (d, 2 H, 2′-H and 6′-H), 7.95 (m, 2 H, quinazoline 8-H and 7-H), 8.09 (d, 1H, quinazoline 5-H).

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]glycine (4). Method B.** The methyl ester **36** (180 mg, 0.43 mmol) was stirred for 1 h in a mixture of 1:1 aqueous EtOH (17 mL) and 1 N aqueous NaOH (1.0 mL). The resulting solution was concentrated under vacuum to ca. 5 mL, filtered into a centrifuge tube, and acidified with 2 N aqueous HCl to pH 3.0. The resulting gelatinous precipitate was isolated by centrifugation, washed (4×) with H_2O, and freeze-dried to yield an amorphous off-white solid: 90 mg (48%); mp 240–250 °C dec; NMR (Me_2SO-d_6) δ 2.32 (s, 3 H, CH_3), 3.19 (t, 1 H, C≡CH), 3.81 (d, 2 H, $CONHCH_2$), 4.32 (d, 2 H, $CH_2C{\equiv}C$), 4.76 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, 3′-H and 5′-H), 7.54 (d, 1 H, quinazoline 8-H), 7.69 (dd, 1H, quinazoline 7-H), 7.70 (d, 2 H, 2′-H and 6′-H), 7.98 (d, 1 H, quinazoline 5-H), 8.33 (d, 1H, CONH). Anal. ($C_{22}H_{20}N_4O_4 \cdot 1.5H_2O$) C, H, N.

***tert*-Butyl-4-(Prop-2-ynylamino)benzoate (37).** *tert*-Butyl 4-aminobenzoate was prepared by the literature method[39] or more conveniently by treating 4-nitrobenzoyl chloride with excess *tert*-butyl alcohol and pyridine in toluene followed by hydrogenation of the resulting *tert*-butyl 4-nitrobenzoate over 10% Pd/C in EtOAc.

A mixture of *tert*-butyl 4-aminobenzoate (10.5 g, 54 mmol), propargyl bromide (7.3 mL of an 80% w/v solution in toluene, 65.5 mmol), and powdered K_2CO_3 (7.5 g, 54 mmol) in DMA (85 mL) was stirred at 50 °C for 24 h. The cooled reaction mixture was filtered, and the filtrate was evaporated to a brown oil. This was purified by chromatography using 6:1 v/v hexane/EtOAc as eluent to yield a white solid: mp 87–88 °C; NMR (Me_2SO-d_6) δ 1.50 (s, 9 H, Bu^t), 3.06 (t, 1 H, C≡CH), 3.92 (dd, 2 H, $CH_2C{\equiv}C$), 6.63 (d, 2 H, 3-H and 5-H), 6.72 (t, 1 H, NH), 7.66 (d, 2 H, 2-H and 6-H). Anal. ($C_{14}H_{17}NO_2$) C, H, N.

4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic Acid (39). A mixture of **37** (7.3 g, 31.6 mmol), 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline (**34**)[6] (8.0 g, 31.6 mmol), and $CaCO_3$ (3.2 g, 32 mmol) in DMF (100 mL) was stirred for 65 h and filtered, and the filtrate was evaporated. The crude product was purified by chromatography using EtOAc as eluent. The resulting *tert*-butyl ester (2.50 g, 6.2 mmol) was dissolved in CF_3CO_2H (25 mL). After 10 min the CF_3CO_2H was evaporated, and the gummy residue was triturated with ether. The resulting yellow solid was filtered off and dried in vacuo to yield **39** as its CF_3CO_2H salt (2.50 g, 87%): NMR (Me_2SO-d_6) δ 2.48 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 4.36 (d, 2H, $CH_2C{\equiv}C$), 4.84 (br s, 2 H, $ArCH_2N<$), 6.84 (d, 2 H, 3′-H and 5′-H), 7.65 (d, 1 H, quinazoline 8-H), 7.75 (d, 2 H, 2′-H and 6′-H), 7.80 (dd, 1 H, quinazoline 7-H), 8.01 (d, 1H, quinazoline 5-H).

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-alanine (5). Method C.** DPPA (670 μL, 3.11 mmol) and Et_3N (670 μL, 4.78 mmol) were added successively to a stirred solution of **39**·CF_3CO_2H salt (500 mg, 1.08 mmol) and L-alanine ethyl ester hydrochloride (270 mg, 1.76 mmol) in DMF (20 mL) at 0 °C. The mixture was stirred at 0 °C for 5 h and then at ambient temperature for 48 h and poured into ice/H_2O (100 mL). The precipitated solid was isolated by centrifugation, washed with water, and dried under vacuum. This crude product was purified by chromatography using 24:1 v/v CH_2Cl_2/EtOH as eluent to yield **40** as a foam: 115 mg (24%); NMR (Me_2SO-d_6) δ 1.16 (t, 3 H, CH_2CH_3), 1.36 (d, 3 H, $CHCH_3$), 2.30 (s, 3 H, CH_3), 3.18 (t, 1 H, C≡CH), 4.09 (q, 2 H, CH_2CH_3), 4.31 (d, 2 H, $CH_2C{\equiv}C$), 4.40 (m, 1 H, $CHCH_3$), 4.78 (br s, 2 H, $ArCH_2N<$), 6.84 (d, 2 H, 3′-H and 5′-H), 7.54 (d, 1 H, quinazoline 8-H), 7.68 (dd, 1H, quinazoline 7-H), 7.70 (d, 2 H, 2′-H and 6′-H), 7.98 (d, 1 H, quinazoline 5-H), 8.35 (d, 1H, CONH).

This ester (110 mg, 0.25 mmol) was stirred for 2 h in a mixture of 1 N aqueous NaOH (640 μL, 0.64 mmol), EtOH (4 mL), and H_2O (4 mL). The clear solution was evaporated to ca. 2 mL, filtered into a centrifuge tube, and acidified to pH 3.0 with 2 N aqueous HCl. The precipitate was isolated by centrifugation, washed (4×) with H_2O, and vacuum-dried to give an amorphous white solid: 85 mg (82%); mp 165–170 °C; NMR (Me_2SO-d_6) δ 1.32 (d, 3 H, CH_3), 2.31 (s, 3 H, CH_3), 3.18 (t, 1 H, C≡CH), 4.30 (m, 3 H, $NHCH$ and $CH_2C{\equiv}C$), 4.78 (s, 2 H, $ArCH_2N<$), 6.83 (d, 2 H, 3′-H and 5′-H), 7.52 (d, 1 H, quinazoline 8-H), 7.68 (dd, 1H, quinazoline 7-H), 7.72 (d, 2 H, 2′-H and 6′-H), 7.96 (d, 1 H, quinazoline 5-H), 8.21 (d, 1H, CONH), 12.13 (s, 1 H, NH); MS (FAB) *m/z* 418 $[M - H]^-$. Anal. ($C_{23}H_{22}N_4O_4 \cdot H_2O$) C, H; N: calcd, 12.8; found, 12.3.

4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl Azide (41). Et_3N (9.84 mL, 70 mmol) and DPPA (6.10 mL, 28.3 mmol) were added successively to a stirred solution of **39**·CF_3CO_2H salt (8.0 g, 17.35 mmol) in DMF (60 mL), and stirring was continued for 18 h. The resulting precipitated solid was isolated by centrifugation, washed with DMF (3 × 40 mL) and

Et_2O (40 mL), and vacuum-dried: 5.3 g (82%); mp 215–218 °C dec; NMR (Me_2SO-d_6) δ 2.32 (s, 3 H, CH_3), 3.23 (t, 1 H, C≡CH), 4.39 (d, 2 H, $CH_2C{\equiv}C$), 4.85 (br s, 2 H, $ArCH_2N<$), 6.88 (d, 2 H, 3′-H and 5′-H), 7.53 (d, 1 H, quinazoline 8-H), 7.67 (dd, 1 H, quinazoline 7-H), 7.78 (d, 2 H, 2′-H and 6′-H), 7.94 (d, 1 H, quinazoline 5-H), 12.14 (b s, 1 H, NH); MS (FAB) *m/z* 373 $[MH]^+$. Anal. ($C_{20}H_{16}N_6O_2{\cdot}0.33H_2O$) C, H, N.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-D,L-2-(4-fluorophenyl)glycine (13). Method D.** D,L-2-(4-Fluorophenyl)glycine (187 mg, 1.11 mmol) and DBU (600 μL, 4.03 mmol) were added successively to a stirred suspension of **41** (372 mg, 1.0 mmol) in DMF (15 mL). Stirring was continued in the absence of light for 20 h. The solvent was evaporated under reduced pressure, and the residue was treated with ice-cold H_2O (20 mL). The resulting solution was acidified to pH 3.0 with 2 N aqueous HCl. The precipitated solid was filtered off, washed with H_2O, and vacuum-dried: 330 mg (66%); mp 197–202 °C; NMR (Me_2SO-d_6) δ 2.34 (s, 3 H, CH_3), 3.20 (t, 1 H, C≡CH), 4.33 (d, 2 H, $CH_2C{\equiv}C$), 4.77 (br s, 2 H, $ArCH_2N<$), 5.48 (d, 1 H, CONHC*H*), 6.84 (d, 2 H, 3′-H and 5′-H), 7.15 (dd, 2 H, ArH), 7.42–7.60 (m, 3 H, quinazoline 8-H and ArH), 7.68 (dd, 1H, quinazoline 7-H), 7.77 (d, 2 H, 2′-H and 6′-H), 7.97 (d, 1 H, quinazoline 5-H), 8.54 (d, 1 H, CONH), 12.54 (broad s, 1 H, NH); MS (FAB) *m/z* 497 $[M - H]^-$. Anal. ($C_{23}H_{22}FN_4O_4{\cdot}1.75H_2O$) C, H, N.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-*N*-ethylamine (16). Method E.** To a stirred solution of **39**·CF_3CO_2H salt (520 mg, 1.13 mmol) in DMF (10.6 mL) at 0 °C was added Et_3N (460 μL, 3.29 mmol), $EtNH_2$ (115 μL, 1.8 mmol), and DPPA (355 μL, 1.65 mmol). Stirring was continued for 5 h at 0 °C and then for 16 h at ambient temperature. The solution was poured into ice/H_2O (100 mL). The precipitated solid was isolated by centrifugation, washed (4×) with H_2O, and vacuum-dried: 161 mg (29%); mp 245–249 °C; NMR (Me_2SO-d_6) δ 1.07 (t, 3 H, CH_2CH_3), 2.33 (s, 3 H, CH_3), 3.17 (m, 2 H, $NHCH_2$), 3.20 (t, 1 H, C≡CH), 4.28 (d, 2 H, $CH_2C{\equiv}C$), 4.76 (br s, 2 H, $ArCH_2N<$), 6.83 (d, 2 H, 3′-H and 5′-H), 7.54 (d, 2 H, quinazoline 8-H), 7.68 (d, 2 H, 2′-H and 6′H), 7.70 (dd, 1H, quinazoline 7-H), 7.96 (d, 1 H, quinazoline 5-H), 8.08 (t, 1 H, CONH). Anal. ($C_{22}H_{22}N_4O_2{\cdot}0.5H_2O$) C, H, N.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamine (3). Method F.** The carboxylic acid **39**·CF_3CO_2H salt (462 mg, 1.0 mmol) was condensed with L-glutamine *tert*-butyl ester hydrochloride according to method E. The solvent was evaporated, and the residue was triturated with ice/H_2O (50 mL). The white solid crude product was isolated by centrifugation, washed with H_2O, and vacuum-dried. The crude product was dissolved in CF_3CO_2H (10 mL), and after 1 h the CF_3CO_2H was removed by rotary evaporation. The gummy residue was dissolved in the minimum volume of DMSO (ca. 500 μL), and this solution was diluted with a mixture of 54.9:44.9:0.2% v/v MeOH/H_2O/CF_3CO_2H (12 mL). This solution was chromatographed on a DYNAMAX-60A ODS column (21.4 mm i.d. × 25 cm *L*), eluting with the same solvent mixture and collecting 10 mL fractions. Fractions containing pure product by HPLC were pooled, and the MeOH was removed by rotary evaporation. The resulting aqueous solution was lyophilized to give an amorphous white solid: 150 mg (30%); mp 155–157 °C; NMR (Me_2SO-d_6) δ 1.97 (m, 2 H, $CHCH_2CH_2CO$), 2.17 (t, 2 H, $CHCH_2CH_2CO$), 2.32 (s, 3 H, CH_3), 3.18 (t, 1 H, C≡CH), 4.27 (m, 1 H, CONHC*H*), 4.31 (d, 2 H, $CH_2C{\equiv}C$), 4.76 (br s, 2 H, $ArCH_2N<$), 6.84 (d, 2 H, 3′-H and 5′-H), 7.53 (d, 1 H, quinazoline 8-H), 7.68 (dd, 1H, quinazoline 7-H), 7.74 (d, 2 H, 2′-H and 6′-H), 7.96 (d, 1 H, quinazoline 5-H), 8.33 (d, 1 H, CONH); MS (FAB) *m/z* 476 $[MH]^+$. Anal. ($C_{25}H_{25}N_5O_5{\cdot}1.5H_2O$) C, H, N.

4-[*N*-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoic Acid (44). A mixture of **37** (16.0 g, 69.3 mmol), the bromomethyl compound **42**[40] (80.0 g, 0.21 mol), and 2,6-lutidine (10 mL, 86 mmol) in DMA (450 mL) was stirred at 55 °C for 16 h under argon. The mixture was poured into H_2O (400 mL) and extracted with CH_2Cl_2 (2 × 300 mL). The combined CH_2Cl_2 solutions were washed with H_2O, dried, and evaporated. The crude product was purified by chromatography using a gradient of 7:3 to 1:1 v/v hexane/EtOAc as eluent. The purified product **43** was dissolved in CF_3CO_2H (400 mL). After 40 min the CF_3CO_2H was evaporated, and the residue was purified by chromatography using a gradient of 9:1 to 4:1 v/v hexane/acetone to yield **44** as an off-while solid: 26.3 g (82%); mp 188–189 °C; NMR (Me_2SO-d_6) δ 1.14 (s, 9 H, Bu^t), 2.59 (s, 3 H, CH_3), 3.20 (t, 1 H, C≡CH), 4.35 (d, 2 H, $CH_2C{\equiv}C$), 4.81 (br s, 2 H, $ArCH_2N<$), 6.04 (s, 2 H, OCH_2N), 6.83 (d, 2 H, 3′-H and 5′-H), 7.57 (d, 2 H, quinazoline 8-H), 7.74 (dd, 1 H, quinazoline 7-H), 7.75 (d, 2 H, 2′-H and 6′-H), 8.10 (d, 1 H, quinazoline 5-H); MS (FAB) *m/z* 462 $[MH]^+$. Anal. ($C_{26}H_{27}N_3O_5$) C, H, N.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-3-(4-nitrophenyl)alanine (10). Method G.** Oxalyl chloride (146 μL, 1.67 mmol) was added over 5 min to a cold (0–5 °C), stirred mixture of **44** (500 mg, 1.08 mmol) and CH_2Cl_2 (12 mL) containing DMF (5 drops). The pale yellow mixture was stirred below 5 °C for 2 h, and the solvent was removed by rotary evaporation below 20 °C. The residue **45** was suspended in CH_2Cl_2 (15 mL), and a mixture of Et_3N (468 μL, 3.37 mmol) and L-3-(4-nitrophenyl)alanine methyl ester hydrochloride (309 mg, 1.38 mmol) was added. The mixture was stirred for 16 h. The resulting solution was diluted with CH_2Cl_2 (40 mL), washed with H_2O (3 × 20 mL), dried, and evaporated. The residue (crude **46**) was purified by chromatography using a gradient of EtOAc in hexane as eluent.

The purified methyl ester **46** (381 mg, 0.57 mmol) was stirred for 2.5 h in a mixture of 1 N aqueous NaOH (5.6 mL, 5.6 mmol) and EtOH (10 mL). The EtOH was evaporated, and the resulting aqueous solution was filtered and acidified with 2 N aqueous HCl to pH 2.0. The precipitated solid was filtered off, washed with H_2O, and vacuum-dried: 256 mg (79%); mp 234–237 °C; NMR (Me_2SO-d_6) δ 2.34 (s, 3H, CH_3), 3.10–3.38 (m, 3 H, C≡CH and $CHCH_2$), 4.29 (d, 2 H, $CH_2C{\equiv}C$), 4.63 (m, 1 H, $CHCH_2$), 4.76 (br s, 2 H, $ArCH_2N<$), 6.82 (d, 2 H, 3′-H and 5′-H), 7.48–7.72 (m, 6 H, quinazoline 8-H and 7-H, 2′-H and 6′-H, and benzyl 2-H and 6-H), 7.96 (d, 1 H, quinazoline 5-H), 8.12 (d, 2 H, benzyl 3-H and 5-H), 8.33 (d, 1 H, CONH), 12.12 (broad s, 1 H, NH); MS (FAB) *m/z* 540 $[MH]^+$. Anal. ($C_{29}H_{25}N_5O_6{\cdot}1.75H_2O$) C, H, N.

2,6,7-Trimethyl-4*H*-3,1-benzoxazinone (57). Powdered 2-acetamido-4,5-dimethylbenzoic acid (**56**)[25] (305 g, 1.61 mol) was added with stirring to hot xylene (1.5 L). Xylene (ca. 800 mL) was distilled from the mixture at atmospheric pressure to remove traces of H_2O, and then acetic anhydride (300 mL) was added dropwise over 30 min to the refluxing mixture. Stirring under reflux was continued for 3 h, and then a further ca. 700 mL of solvent was removed by distillation. The reaction mixture was allowed to cool to room temperature overnight with continued stirring. The precipitated brown solid was filtered off, washed with *n*-hexane, and dissolved in CH_2Cl_2. This solution was treated with carbon, passed through a short column of Kieselgel 60, and evaporated to dryness. The resulting pale brown solid was triturated with *n*-hexane, filtered off, and vacuum-dried. The product (116 g, 38%) was used without further purification in the preparation of **58**.

3,4-Dihydro-4-oxo-2,6,7-trimethylquinazoline (58). A mixture of **57** (400 g, 2.12 mol), 35% aqueous ammonia (sp. gr 0.88, 2.5 L, ca. 45 mol), and 2 N aqueous NaOH (150 mL, 0.3 mol) was stirred in a Büchi pressure vessel at 60 °C for 16 h. The reaction mixture was cooled to room temperature and neutralized with HOAc. The resulting white solid precipitate was filtered off, washed with H_2O, and vacuum-dried: 371 g (93%); mp 288–293 °C dec; NMR (Me_2SO-d_6) δ 2.62 (s, 3 H, CH_3), 2.64 (s, 3 H, CH_3), 2.66 (s, 3 H, CH_3) 7.33 (s, 1 H, quinazoline 8-H), 7.80 (s, 1 H, quinazoline 5-H).

3,4-Dihydro-4-oxo-3-[(pivaloyloxy)methyl]-2,6,7-trimethylquinazoline (59). A suspension of **58** (200 g, 1.06 mol) in DMF (1.2 L) was stirred under argon at 0 °C. Sodium hydride (51.2 g of a 60% w/w dispersion in oil, 1.28 mol) was added in portions while keeping the temperature below 5 °C. Stirring was continued for 1 h, and then chloromethyl pivalate

(184 mL, 1.17 mol) was added dropwise over 25 min, again keeping the temperature below 5 °C. The reaction mixture was stirred for a further 30 min below 5 °C and for 60 h at room temperature. It was then cooled back to 0 °C and treated dropwise over 1 h with 1 N aqueous HCl (500 mL). The resulting thick slurry was stirred at 0 °C for 1 h and filtered. The filtercake was washed with H_2O (6 L) and hexane (2 L) and vacuum-dried at 50 °C for 48 h to give an off-white solid: 247 g (77%); mp 152–161 °C; NMR (Me_2SO-d_6) δ 1.22 (s, 9 H, Bu^t), 2.39 (s, 3 H, CH_3), 2.42 (s, 3 H, CH_3), 2.72 (s, 3 H, CH_3), 6.12 (s, 2 H, OCH_2N), 7.53 (s, 1 H, quinazoline 8-H), 8.01 (s, 1 H, quinazoline 5-H).

6-(Bromomethyl)-3,4-dihydro-4-oxo-3-[(pivaloyloxy)methyl]-2,7-dimethylquinazoline (60). A mixture of **59** (311 g, 1.03 mol), NBS (199 g, 1.12 mol), and azobisisobutyronitrile (8 g, 0.05 mol) in CCl_4 (4 L) was stirred under reflux for 4 h to give a clear orange solution. On cooling, an off-white precipitate was obtained which was filtered off and washed successively with alternate portions of CH_2Cl_2 and H_2O until all the material had dissolved between the phases. The combined organic phase was washed with H_2O, dried, and evaporated to dryness. The residue was triturated with hexane to give a white solid which was purified by chromatography using a gradient of 0–15% v/v EtOAc in CH_2Cl_2 as eluent. The product was isolated as a white solid: 223 g, 57%; mp 142–148 °C; NMR ($CDCl_3$) δ 1.21 (s, 9 H, Bu^t), 2.56 (s, 3 H, CH_3), 2.63 (s, 3 H, CH_3), 4.59 (s, 2 H, CH_2Br), 6.09 (s, 2 H, OCH_2N), 7.44 (s, 1 H, quinazoline 8-H), 8.19 (s, 1 H, quinazoline 5-H). Anal. ($C_{17}H_{21}BrN_2O_3$) N, H; C: calcd, 53.6; found, 54.1, Br: calcd, 21.0; found, 20.5.

4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic Acid (62). A mixture of **67** (66.2 g, 0.27 mol), the bromomethyl compound **60** (85 g, 0.22 mol) and 2,6-lutidine (29.3 mL, 0.25 mol) in DMF (600 mL) was stirred at 95 °C for 8 h under argon. The cooled mixture was poured into H_2O (1.6 L) and extracted with EtOAc (2 × 1 L). The combined EtOAc extracts were washed with H_2O and dried, and the solvent was evaporated to give a beige solid. The solid was washed with hexane, dried in under vacuum, and purified by chromatography using a gradient of 0–10% v/v EtOAc in CH_2Cl_2 as eluent. The purified product **61** (85 g, 69%) was dissolved in CF_3CO_2H (600 mL). After 1 h the deep purple solution was poured into H_2O and extracted with EtOAc (2 × 500 mL). The combined organic solutions were washed with H_2O, dried, and evaporated to dryness. The viscous gummy residue was triturated with 1:1 EtOAc/ether to give an off-white solid which was air-dried overnight. 65 g (85%); mp 227–228 °C; NMR (Me_2SO-d_6) δ 1.13 (s, 9 H, Bu^t), 2.46 (s, 3 H, CH_3), 2.59 (s, 3 H, CH_3), 3.24 (t, 1 H, C≡CH), 4.32 (d, 2 H, $CH_2C{\equiv}C$), 4.74 (br s, 2 H, $ArCH_2N<$), 6.01 (s, 2 H, OCH_2N), 6.59 (dd, 1 H, 3′-H), 6.64 (dd, 1 H, 5′-H), 7.49 (s, 1 H, quinazoline 8-H), 7.72 (dd, 1 H, 6′-H), 7.73 (s, 1 H, quinazoline 5-H). Anal. ($C_{27}H_{28}FN_3O_5 \cdot 1.25CF_3CO_2H$) C, H, N.

Pentafluorophenyl 4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoate (63). A mixture of acid **62**·1.25CF_3CO_2H salt (65 g, 0.102 mol), pentafluorophenol (19.7 g, 0.107 mol), dicyclohexylcarbodiimide (44 g, 0.214 mol), Et_3N (15 mL, 0.108 mol), and 4-pyrrolidinopyridine (300 mg, 2 mmol) in EtOAc was stirred overnight under argon. The mixture was filtered through a bed of Kieselgel 60, which was washed with EtOAc. The combined filtrates were evaporated to give a brown oil which gave an off-white solid on trituration with hexane. This crude product was purified by chromatography using 4:4 v/v CH_2Cl_2/EtOAc as eluent to yield a white solid: 60 g (69%); mp 181 °C; NMR (Me_2SO-d_6) δ 1.12 (s, 9 H, Bu^t), 2.47 (s, 3 H, CH_3), 2.58 (s, 3 H, CH_3), 3.29 (t, 1 H, C≡CH), 4.42 (d, 2 H, $CH_2C{\equiv}C$), 4.85 (br s, 2 H, $ArCH_2N<$), 6.00 (s, 2 H, OCH_2N), 6.78 (br dd, 2 H, 3′-H and 5′-H), 7.50 (s, 1 H, quinazoline 8-H), 7.72 (s, 1 H, quinazoline 5-H), 7.95 (dd, 1 H, 6′-H). Anal. ($C_{33}H_{27}F_6N_3O_5$) C, H, N.

***tert*-Butyl 2-Fluoro-4-nitrobenzoate (65).** Toluene-*p*-sulfonyl chloride (1.8 kg, 9.5 mol) was added in portions to a stirred suspension of 2-fluoro-4-nitrobenzoic acid **64**[7] (1.04 kg, 5.6 mol) in pyridine (5.6 L) while maintaining the temperature at 15 °C with ice bath cooling. After 5 min, *tert*-butyl alcohol (775 mL, 8.3 mol) was added over 5 min. An exotherm was produced which raised the temperature to 27 °C. The resulting mixture was stirred at room temperature for 2.5 h and poured into H_2O (30 L). The solid precipitate was filtered off, washed with H_2O, and vacuum-dried at 40 °C: 1.26 kg (93%); mp 79 °C; NMR ($CDCl_3$) δ 1.62 (s, 9 H, Bu^t), 7.98 (dd, 1 H, Ar 6-H), 8.04 (m, 2 H, Ar 3-H and 5-H).

***tert*-Butyl 4-Amino-2-fluorobenzoate (66).** The nitro compound **65** (602 g, 2.5 mol) in EtOAc (5 L) was hydrogenated for 20 h at 30 °C at a pressure of 30 bar in the presence of 10% Pd/C (6 g) in a 7 L Parr vessel. The vessel was flushed with N_2, and the solution was filtered through Celite to remove the catalyst. The solvent was evaporated, and the crude orange solid product was triturated with hexane, filtered off, washed with more hexane, and vacuum-dried at 35 °C to produce a pale yellow solid: 518 g (99%); mp 103 °C; NMR ($CDCl_3$) δ 1.58 (s, 9 H, Bu^t), 4.13 (br s, 2 H, NH_2), 6.31 (dd, 1 H, Ar 3-H), 6.39 (dd, 1 H, Ar 5-H), 7.69 (dd, 1 H, Ar 6-H). Anal. ($C_{11}H_{14}FNO_2$) C, H, N.

***tert*-Butyl 2-Fluoro-4-(prop-2-ynylamino)benzoate (67).** A mixture of **66** (1.38 kg, 6.54 mol), propargyl bromide (800 mL of an 80% w/w solution in toluene, 7.18 mol), and 2,6-lutidine (1.5 L, 12.9 mol) in DMF (6 L) was stirred under argon at 95 °C for 24 h. Most of the solvent was removed by rotary evaporation at 40 °C, and the viscous black residue was partitioned between EtOAc and H_2O. The organic solution was washed with H_2O, treated with carbon, dried, and evaporated to dryness. The crude viscous black oil was purified by chromatography in five batches using 4:1 v/v CH_2Cl_2/hexane as eluent to yield an off-white solid: 1.032 kg (63%); mp 112 °C; NMR (Me_2SO-d_6) δ 1.49 (s, 9 H, Bu^t), 3.12 (t, 1 H, C≡CH), 3.94 (d, 2 H, $CH_2C{\equiv}C$), 6.39 (dd, 1 H, Ar 3-H), 6.48 (dd, 1 H, Ar 5-H), 7.57 (dd, 1 H, Ar 6-H). Anal. ($C_{14}H_{16}FNO_2$) C, H, N.

***N*-[4-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-*tert*-leucine (68). Method H.** A mixture of the pentafluorophenyl ester **63** (392 mg, 0.6 mmol), L-*tert*-leucine (105 mg, 0.8 mmol), Et_3N (337 μL, 2.4 mmol), and 1-hydroxybenzotriazole hydrate (25 mg) in DMA (6 mL) was stirred for 16 h under argon to give a clear almost colorless solution. The solvent was evaporated below 40 °C, and the residue was partitioned between EtOAc (2 × 25 mL) and H_2O (15 mL), acidified to pH 1 by the addition of a few drops of 2 N aqueous HCl. The combined organic solutions were washed with H_2O, dried, and evaporated. Traces of DMA were removed from the yellow gummy residue by the rotary evaporation of added xylene prior to purification by chromatography using a gradient of 0–8% v/v EtOH in CH_2Cl_2 as eluent to yield a colorless foam: 248 mg (68%); NMR (Me_2SO-d_6) δ 0.99 (s, 9 H, Bu^t), 1.12 (s, 9 H, Bu^t), 2.45 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 4.29 (d, 1 H, NHC*H*), 4.31 (d, 2 H, $CH_2C{\equiv}C$), 4.72 (s, 2 H, CH_2N), 6.00 (s, 2 H, OCH_2N), 6.63 (dd, 1 H, 3′-H), 6.67 (dd, 1 H, 5′-H), 7.40 (s, 1 H, quinazoline 8-H), 7.56 (dd, 1 H, 6′-H), 7.76 (s, 1 H, quinazoline 5-H); MS (FAB) *m*/*z* 607 $[MH]^+$.

***N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-*tert*-leucine (49).** The (pivaloyloxy)methyl compound **68** (238 mg, 0.39 mmol) was stirred under argon for 16 h in a mixture of 1 N aqueous NaOH (2.40 mL, 2.40 mmol) and EtOH (10 mL) to give a clear solution. The EtOH was evaporated, and the resulting aqueous solution was filtered into a centrifuge tube and acidified with 2 N aqueous HCl to pH 3.0. The precipitated solid was isolated by centrifugation, washed (3×) with H_2O, and vacuum-dried: 152 mg (78%); mp 258–260 °C dec; NMR (Me_2SO-d_6) δ 0.99 (s, 9 H, Bu^t) 2.31 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 4.30 (d, 2 H, $CH_2C{\equiv}C$), 4.32 (d, 1 H, NHC*H*), 4.69 (s, 2 H, CH_2N) 6.62 (dd, 1 H, 3′-H), 6.67 (dd, 1 H, 5′-H), 7.43 (s, 1 H, quinazoline 8-H), 7.55 (dd, 1 H, 6′-H), 7.70 (s, 1 H, quinazoline 5-H); MS (FAB) *m*/*z* 493 $[MH]^+$. Anal. ($C_{27}H_{29}FN_4O_4$) C, H, N.

2-[*N*-[4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-(pivaloyloxymethyl)-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]aminomethyl]thiazole (69). A mixture of the pentafluorophenyl ester **63** (330 mg, 0.5 mmol), 2-

aminomethylthiazole.2HCl (130 mg, 0.7 mmol), Et_3N (393 μL, 2.8 mmol) and 1-hydroxybenzotriazole hydrate (10 mg) in DMA (5 mL) was stirred for 16 h under argon to give a clear almost colourless solution. The solvent was evaporated below 40 °C and the residue was partitioned between EtOAc (2 × 25 mL) and H_2O (15 mL). The combined organic solutions were washed with H_2O, dried and evaporated. Traces of DMA were removed from the brown gummy residue by the rotary evaporation of added xylene prior to purification by chromatography using a gradient of 0–5% v/v EtOH in CH_2Cl_2 as eluent to give a white foam: 208 mg (71%); NMR (Me_2SO-d_6) δ 1 12 (s, 9 H, Bu^t), 2.46 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 4.31 (d, 2 H, CH_2C≡C), 4.70 (d, 2 H, NHCH_2), 4.72 (s, 2 H, CH_2N), 6.00 (s, 2 H, OCH_2N), 6.65 (dd, 1 H, 3′-H), 6.69 (dd, 1 H, 5′-H), 7.47 (s, 1 H, quinazoline 8-H), 7.58 (d, 1 H, thiazole 5-H), 7.60 (dd, 1 H, 6′-H), 7.71 (d, 1 H, thiazole 4-H), 7.76 (s, 1 H, quinazoline 5-H), 8.67 (m, 1 H, CONH).

2-[[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]methyl]thiazole (54). Method I. The (pivaloyloxy)-methyl compound **69** (192 mg, 0.326 mmol) was stirred under argon in a saturated solution of NH_3 in MeOH (15 mL). A clear solution was obtained initially, but after ca. 30 min a white precipitate began to appear. Stirring was continued for 16 h, the precipitate was filtered off, washed with MeOH, and vacuum-dried: 125 mg (80%); mp 270–271 °C; NMR (Me_2SO-d_6) δ 2.31 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 4.30 (d, 2 H, CH_2C≡C), 4.69 (s, 2 H, CH_2N), 4.71 (d, 2 H, NHCH_2), 6.64 (dd, 1 H, 3′-H), 6.68 (dd, 1 H, 5′-H), 7.43 (s, 1 H, quinazoline 8-H), 7.59 (d, 1 H, thiazole 5-H), 7.60 (dd, 1 H, 6′-H), 7.70 (s, 1 H, quinazoline 5-H), 7.71 (d, 1 H, thiazole 4-H), 8.68 (m, 1 H, CONH); MS (FAB) m/z 476 $[MH]^+$. Anal. ($C_{25}H_{22}FN_5O_2S$·H_2O) C, H, N.

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