Quinazoline Antifolates Thymidylate Synthase Inhibitors: Lipophilic Analogues with Modification to the C2-Methyl Substituent

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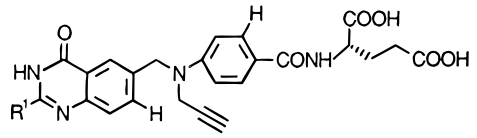
Modification of the potent thymidylate synthase (TS) inhibitor 1-[[*N*-[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]amino]methyl]-3-nitrobenzene (**4a**) has led to the synthesis of quinazolinone antifolates bearing functionalized alkyl substituents at C2. A general synthetic route was developed which involved coupling the appropriate 1-[[*N*-[4-(alkylamino)benzoyl)amino]methyl]-3-nitrobenzene **20**−**22** with a 6-(bromomethyl)-2-(acetoxymethyl)-3,4-dihydro-4-oxoquinazoline **9** or **10**. Replacement of the 2-acetoxy group by a chlorine atom followed by the displacement of the halogen of **25a**−**c** by various nucleophiles led to compounds **26**−**40**. Good TS (IC_{50} <1 μM) and growth inhibition (IC_{50} 0.1−1 μM) were found with most of these new antifolates. TS inhibitors in this series do not apparently require the reduced folate carrier (RFC) for cell entry (they most likely penetrate the cell membrane by passive diffusion) and are not polyglutamated. N, O, S, Cl, and CN as well as large amino and mercapto substituents were tolerated by the enzyme. The simultaneous incorporation of 7-methyl and 2′-F substituents gave a series of highly potent agents inhibiting cell growth at concentrations <1 μM (**24**, **27bc**; **30**−**32b**, **35b**). The incorporation of suitable C2 substituents has overcome the decrease in aqueous solubility observed with lipophilic quinazoline antifolates. This is best illustrated by compound **31a**, where up to a 54-fold increase in solubility has been achieved by the incorporation of an *N*-methylpiperazine nucleus into the C2-methyl group of **4a**.

Despite being withdrawn from clinical studies because of unpredictable nephrotoxicity, the quinazolinone-based antifolate CB3717 (**1**) established the principle of antitumor chemotherapy with a specific inhibitor of thymidylate synthase (TS) by showing responses in phase I/II[1−5] clinical trials against breast, ovarian, and liver cancers. A search for more soluble analogues[6−11] devoid of renal toxicity led to the discovery of the 2-methylquinazolinone analogue ICI 198583 (**2**)[12] and more recently Zeneca ZD1694 (Tomudex, **3**).[13−15] The latter compound has completed phase I, phase II, and phase III clinical trials.[16−18]

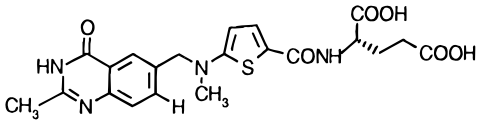
To inhibit tumor cell growth ICI 198583 and ZD1694 require rapid transport into cells by the reduced folate carrier (RFC) and subsequent conversion to polyglutamated forms catalyzed by the enzyme folylpolyglutamate synthetase (FPGS).[12,13,15] The resultant accumulated polyglutamates are up to 100 times more potent as inhibitors of TS than the parent drugs[19,20] and are not readily effluxed from cells.[12,13,21]

The potential for tumor cells to acquire resistance to folate-based antimetabolites[22,23] including ZD1694 by deletion or modification of either the RFC[24] or FPGS[24] prompted the search for new TS inhibitors which require neither of these processes to express cell growth inhibition *in vitro*.

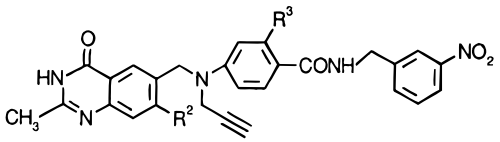
We recently reported the design of molecules that satisfy this profile in which the glutamic acid residue (cf., e.g. 198583) was replaced by lipophilic alkyl, benzyl, and heterocyclic benzyl amides.[25] This early work clearly showed that molecules such as **4a,b** (Scheme 1, Table 1) were excellent inhibitors of the isolated TS enzyme and were growth inhibitory to cells in culture. Nevertheless, because of their relatively high lipophilicity (**4a**, log P = 3.7) and the absence of a potentially



1 $R^1 = NH_2$ CB3717
2 $R^1 = CH_3$ ICI 198583



3 ZD 1694 TOMUDEX



4 a-b
a $R^2 = H$ $R^3 = H$
b $R^2 = CH_3$ $R^3 = F$

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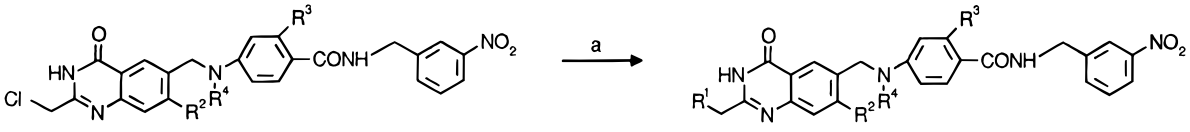
Scheme 1[a]



25 a,b,c

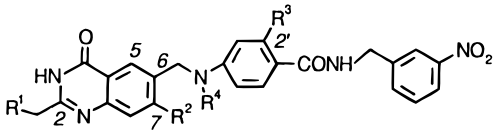
a $R^2 = H$ $R^3 = H$ $R^4 = CH_2C{\equiv}CH$
b $R^2 = CH_3$ $R^3 = F$ $R^4 = CH_2C{\equiv}CH$
c $R^2 = CH_3$ $R^3 = F$ $R^4 = CH_3$

26 $R^1 = O(CH_2)_2OCH_3$
27 $R^1 = N(CH_3)_2$
28 $R^1 = NHCH_3$
29 $R^1 = NH_2$
30 $R^1 = N(CH_3)(CH_2CH_2)N(CH_3)_2$
31 $R^1 = N(CH_2CH_2)_2NCH_3$
32 $R^1 = N(CH_2CH_2)_2O$
33 R1 = N-CH2CH(OH)CH2CH2
34 $R^1 = N(CH_2CH_2)_2OH$
35 R^1 = 2-S-pyrimidine
36 R^1 = 2-S-pyrimidine-4-NH_2
37 R^1 = 2-S-pyrimidine-4-OH
38 R^1 = 2-S-pyrimidine-4-NH_2-6-OH
39 $R^1 = CN$
40 $R^1 = MeSO_2NH$
4 $R^1 = H$ (see ref 25)

See Table I for compounds prepared

[a] (a) **27**−**34a**, method A; **27b**, **30b**, **32b**, **34b**, method B; **27c**, method C; **35a**−**38a**, method D; **35b**, method E; **26a**, method F; **39a**, method G; **40a**, method H.

Table 1. *In Vitro* Activities of C2-Substituted Antifolates

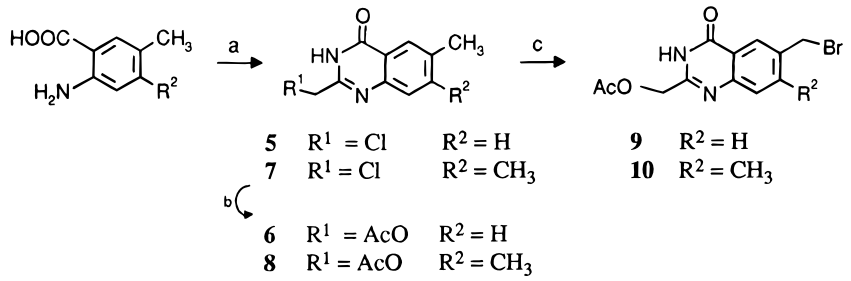


							IC$_{50}$ (μM)			
compd	R^1	R^2	R^3	R^4	mp, °C	formula[a]	inhibn of TS	inhibn of L1210 cell growth in culture	protection at 2/10 IC$_{50}$ % of control growth[b]	L1210:1565 relative resistance ratio[b]
24a	OH	H	H	$CH_2C{\equiv}CH$	201−203	$C_{27}H_{23}N_5O_5{\cdot}2.75H_2O$[c]	0.30	4.8	73/75	
24b	OH	CH_3	F	$CH_2C{\equiv}CH$	254−256[d]	$C_{28}H_{24}FN_5O_5{\cdot}2NaCl$	0.072	0.7	92/71	0.17
24c	OH	CH_3	F	CH_3	166−168	$C_{26}H_{24}FN_5O_5{\cdot}0.5H_2O$	0.08	0.7		
25a	Cl	H	H	$CH_2C{\equiv}CH$	ND1*e*	$C_{27}H_{22}ClN_5O_4$	1.21	2.4	68/6	
26a	$O(CH_2)_2OCH_3$	H	H	$CH_2C{\equiv}CH$	145−148	$C_{30}H_{29}N_5O_6{\cdot}0.66H_2O$	0.64	6.7		
27a	$N(CH_3)_2$	H	H	$CH_2C{\equiv}CH$	ND[e]	$C_{29}H_{28}N_6O_4{\cdot}1.65TFA$	0.62	2.7		
27b	$N(CH_3)_2$	CH_3	F	$CH_2C{\equiv}CH$	136−138[f]	$C_{30}H_{29}FN_6O_4{\cdot}H_2O$	0.14	0.68	93/76	0.76
27c	$N(CH_3)_2$	CH_3	F	CH_3	172−173	$C_{28}H_{29}FN_6O_4{\cdot}0.8H_2O$	0.084	0.58		
28a	$NHCH_3$	H	H	$CH_2C{\equiv}CH$	144[d]	$C_{28}H_{26}N_6O_4{\cdot}1.5TFA$	4.2	7.8		
29a	NH_2	H	H	$CH_2C{\equiv}CH$	ND[e]	$C_{27}H_{24}N_6O_4{\cdot}1.5TFA{\cdot}H_2O$	>1.7	>20		
30a	$N(CH_3)(CH_2CH_2)N(CH_3)_2$	H	H	$CH_2C{\equiv}CH$	ND[e]	$C_{32}H_{35}N_7O_4{\cdot}1.1H_2O$	0.82	5		
30b	$N(CH_3)(CH_2CH_2)N(CH_3)_2$	CH_3	F	$CH_2C{\equiv}CH$	120[d]	$C_{33}H_{36}FN_7O_4{\cdot}0.7CHCl_3$[g]	0.2	0.95		
31a	$N(CH_2CH_2)_2NCH_3$	H	H	$CH_2C{\equiv}CH$	116−138	$C_{32}H_{33}N_7O_4{\cdot}2H_2O$	0.90	5.3	76/6	
31b	$N(CH_2CH_2)_2NCH_3$	CH_3	F	$CH_2C{\equiv}CH$	ND[e]	$C_{33}H_{34}FN_7O_4{\cdot}2H_2O$[h]	0.11	0.58		1.1
32a	$N(CH_2CH_2)_2O$	H	H	$CH_2C{\equiv}CH$	122−128	$C_{31}H_{30}N_6O_5{\cdot}1.2H_2O$	0.38	1.9	87/51	
32b	$N(CH_2CH_2)_2O$	CH_3	F	$CH_2C{\equiv}CH$	146−148[i]	$C_{32}H_{31}FN_6O_5{\cdot}2H_2O$	0.1	0.34	84/73	1.1
33a	$NCH_2CH(OH)CH_2CH_2$	H	H	$CH_2C{\equiv}CH$	148−150	$C_{31}H_{30}N_6O_5{\cdot}1.5H_2O$[j]	0.6	9		
34b	$N(CH_2CH_2OH)_2$	CH_3	F	$CH_2C{\equiv}CH$	192−193	$C_{32}H_{33}FN_6O_6{\cdot}1.5H_2O$	0.16	7.8		
35a	S-2-pyrimidine	H	H	$CH_2C{\equiv}CH$	110−115	$C_{31}H_{25}N_7O_4S{\cdot}0.66H_2O$[k]	0.48	2	85/63	
35b	S-2-pyrimidine	CH_3	F	$CH_2{\equiv}CH$	230−234	$C_{32}H_{26}FN_7O_4S{\cdot}1.75H_2O$	0.11	0.64		0.65
36a	S-2-pyrimidine-4-NH_2	H	H	$CH_2C{\equiv}CH$	130−144	$C_{31}H_{26}N_8O_4S{\cdot}1.5H_2O$	0.84	2.2	81/80	
37a	S-2-pyrimidine-4-OH	H	H	$CH_2C{\equiv}CH$	181−185	$C_{31}H_{25}N_7O_5S{\cdot}1.25H_2O$	3.3	12		
38a	S-2-pyrimidine-4-NH_2-6-OH	H	H	$CH_2C{\equiv}CH$	212−216	$C_{31}H_{26}N_8O_5S{\cdot}2.25H_2O$[l]	1.04	14		
39a	CN	H	H	$CH_2C{\equiv}CH$	ND[e]	$C_{28}H_{22}N_6O_4{\cdot}0.9H_2O$	0.38	11		
40a	$NHSO_2CH_3$	H	H	$CH_2C{\equiv}CH$	ND[e]	$C_{28}H_{26}N_6O_6S{\cdot}0.25TFA$	0.18	>20		
4a[m]	H	H	H	$CH_2C{\equiv}CH$	240−243	$C_{27}H_{23}N_5O_4{\cdot}1.1H_2O$	0.11	1.4	85/60	0.30
4b[m]	H	CH_3	F	$CH_2C{\equiv}CH$	256−258	$C_{28}H_{24}FN_5O_4{\cdot}0.75H_2O$	0.044	0.26	−/66	1.0
4c	H	CH_3	F	CH_3		$C_{26}H_{24}FN_5O_4{\cdot}H_2O$	0.056	0.076	92/83	

[a] Anal. C, H, N except where stated otherwise. [b] See biological evaluation section. [c] N: calcd, 12.80; found, 12.30. [d] Decomposes at this temperature. [e] ND = not determined. [f] Softens >120 °C. [g] N: calcd, 14.06; found, 13.6. [h] N: calcd, 15.14; found, 14.4. [i] Softens >136 °C. [j] N: calcd, 14.16; found, 13.5. [k] N: calcd, 16.25; found, 15.7. [l] N: calcd, 16.90; found, 16.2. [m] See ref 25.

ionized function, the compounds were relatively insoluble at physiological pH. Our attention therefore focused on improving the aqueous solubility of this series of molecules while retaining a submicromolar level of cell growth inhibition *in vitro*. Our previous work[6−8] on analogues of CB3717 modified at the C2 position[26] of the quinazolinone nucleus had given indications that C2-hydroxymethyl derivatives had improved aqueous solubilities compared to their C2-methyl counterparts.[27] These early compounds were generally

Scheme 2[a]



[a] (a) $ClCH_2CN$, MeONa, MeOH; (b) CH_3COONa, DMF; (c) NBS, $CHCl_3$, $(PhCOO)_2$.

micromolar inhibitors of the TS enzyme but had not been investigated further because of poor inhibition of cell growth *in vitro*. This poor activity against the whole cell we believe to be a result from either reduced affinity for the reduced folate carrier (RFC)[28] which in turn may lead to a slow rate of polyglutamate formation. These processes are not relevant to the activity of lipophilic TS inhibitors,[25] but the potential for improved solubility of these quinazolinones by C2 modifications persuaded us to explore this position in our current lipophilic series. This paper now describes the synthesis, solubility properties, and biological activities of analogues of **4a,b** modified at the C2 position of the quinazolinone and compares these new molecules to their glutamate-containing equivalents and C2-methyl analogues.

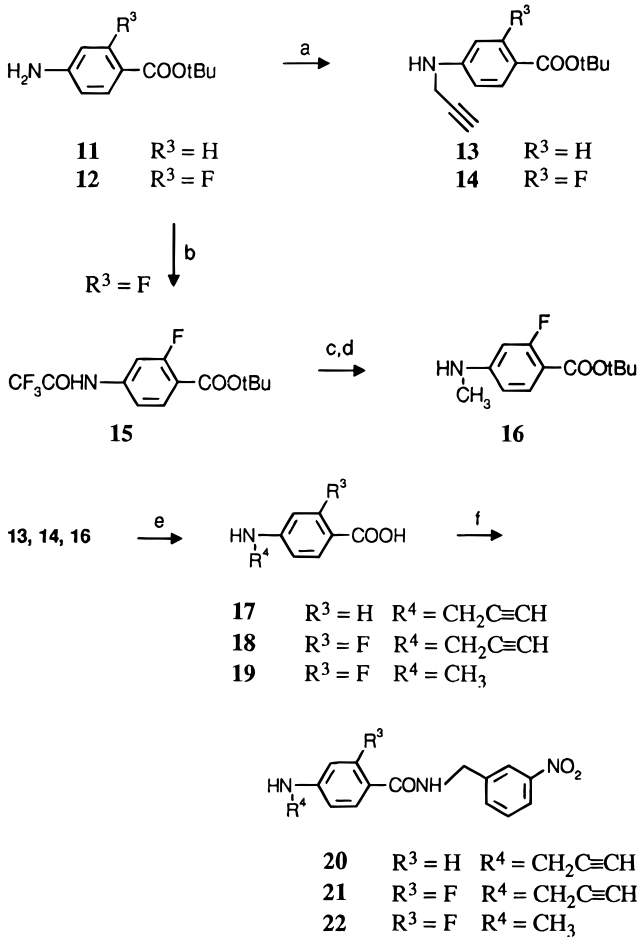
Chemistry

The antifolates modified at the C2 position, listed in Table 1 were prepared by the routes described in Schemes 1 and 4. The strategy adopted involved a late stage displacement of the benzylic halogen of the chloromethyl derivatives **25a**−**c** with a range of nucleophiles either by direct displacement of the chlorine atom with amines in methanol at room temperature in the case of **27**−**34a**−**c** (methods A, B and C) or by reaction with thiolates derived from the corresponding mercaptopyrimidines (as in the case of the 2-[(2-pyrimidinylthio)methyl] antifolate derivatives **35**−**38a**−**c** (methods D and E)). Ether **26a** was obtained by coupling **25a** with sodium 2-methoxyethoxide, generated *in situ* by reaction of 2-methoxyethanol with sodium metal (method F). In preparing the nitrile **39a** the solvent employed was dimethyl sulfoxide (method G). The sulfonamide **40a** was obtained by direct sulfonylation of antifolate **29a** using methanesulfonyl chloride in dichloromethane at room temperature (method H).

The *p*-aminobenzamides **20**−**22** were prepared as described in Scheme 3 or as previously reported.[6,11,25] Acylation of **12** with trifluoroacetic anhydride in dichloromethane in the presence of $CaCO_3$ provided **15**. Treatment of **15** with sodium hydride in DMF followed by alkylation with methyl iodide and deprotection of the aniline function under basic conditions led to **16**. Subsequent cleavage of *tert*-butyl esters **13**, **14**, and **16** was achieved by the classical treatment[25] with CF_3COOH and gave the free acids **17**−**19**. Activation of the acid function with diphenyl phosphorazidate (DPPA) followed by reaction with *m*-nitrobenzylamine provided anilino amides **20**−**22**.

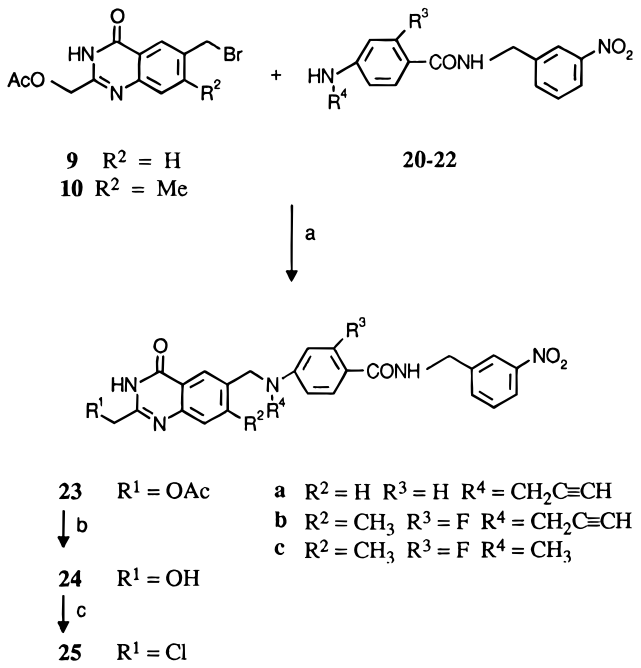
Chloromethyl derivatives **25a**−**c** were crucial intermediates in our strategy, and it was therefore necessary to find an efficient route for their preparation (Schemes 2 and 4). Reaction of 5-methylanthranilic acid or 4,5-dimethylanthranilic acid[2]15 with chloroacetonitrile in methanol in the presence of catalytic amounts of sodium methoxide at 0 °C proved to be an extremely clean reaction when compared to the classical acid conditions[6] and provided the 2-(chloromethyl)quinazolinone **5** and **7** in high yields. Temporary replacement of the chlorine atom of **5** and **7** by an acetoxy group to give **6** and **8** was achieved in almost quantitative yield by using sodium acetate in DMF. Functionalization of the C6 position of the quinazolinones **6** and **8** was achieved by radical bromination of the C6-methyl substituent using *N*-bromosuccinimide (NBS) in refluxing $CHCl_3$ and provided **9** and **10**, respectively. In the case of **10**, regioselective bromination on the C6-methyl was confirmed by the presence of a NOE between H5 and the C6-methylene hydrogens. Compound **10** proved to be

Scheme 3[a]



[a] (a) Propargyl bromide, 2,6-lutidine, DMF, 95 °C; (b) $(CF_3CO)_2O$, Na_2CO_3; (c) Na_2CO_3, MeI, THF; (d) $NaHCO_3$, MeOH, H_2O; (e) CF_3COOH, CH_2Cl_2; (f) DPPA, DMF, Et_3N, *m*-nitrobenzylamine.

Scheme 4[a]



See Table I for compounds prepared

[a] (a) DMF, 2,6-lutidine, 80 °C; (b) 2 N NaOH, MeOH; (c) $SOCl_2$, CH_2Cl_2.

the only monobrominated product of this reaction although minor amounts of the 6,6-dibromo derivative were isolated during the purification. Introduction of the acetoxymethyl function at the C2 position of the quinazolinone allowed us to easily differentiate the methylenes at C2 and C6 during the nucleophilic displacements of the bromine atoms of **9** and **10** with the *p*-aminobenzamides **20**−**22**. This coupling was conducted at 80 °C in DMF in the presence of 2,6-lutidine and gave **23a**−**c** in excellent yields (Scheme 4). After deprotection of the hydroxyl groups, the chloromethyl derivatives **25a**−**c** were generated from **24a**−**c** by treatment at room temperature with $SOCl_2$ in dichloromethane and used directly in the subsequent nucleophilic displacement reactions (methods A, B, C, D, E, and F, Scheme 1).

Biological Evaluation

The compounds prepared are listed in Table 1 and were tested as inhibitors of isolated TS partially purified from L1210 mouse leukaemia cells that overproduce TS.[29] The partial purification and assay method used were as previously described and used a (±)5,10-methylenetetrahydrofolate concentration of 200 μM.[29] 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[30] has acquired resistance to the antitumor antibiotic CI-920.[31] Evidence suggests that this agent enters cells via the reduced folate mechanism and that the L1210:1565 line is resistant due to a very much reduced drug uptake and hence it is cross-resistant to MTX (~200-fold).[32] Both cell lines were grown by suspension culture in RPMI medium without sodium bicarbonate but containing 20 mM HEPES[33] and supplemented with 10% horse serum (L1210) or 10% foetal 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 $\times 10^4$ mL^{-1}. For the thymidine protection experiments the L1210 cells were co-incubated with the compound at concentrations of 2 and 10 times the IC_{50} values and 10 μM thymidine. In Table 1, the thymidine protection results are expressed as percentage of the growth of L1210 cells when grown in a drug-free culture medium and the L1210:1565 relative resistance ratios are calculated as the ratio of L1210:1565 IC_{50} over L1210 IC_{50}. All cell counts were performed with a Model ZM coulter counter. The cell doubling times were 12 (L1210) and 24 h (L1210:1565).

Results and Discussion

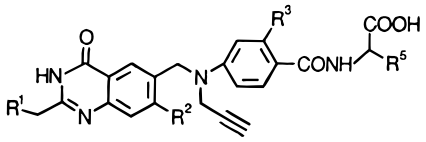
Early data from the glutamic acid series in which substitution of the methyl group at the C2 position of the quinazolinone nucleus led to a minor decrease (2−6-fold) in the potency against the isolated TS enzyme is shown by the comparison of **2** with **41** and **42**[6] (Table 2). Other preliminary data (cf. **24a**, **35a**; and **41**, **42**; Tables 1 and Table 2, respectively) also suggested that replacement of the glutamic acid side chain by the *m*-nitrobenzylamide in these C2-modified analogues resulted in a general retention of inhibitory activity against the TS enzyme.

Analysis of the X-ray structures of CB3717/*E. coli*−TS enzyme ternary complexes[34−37] revealed an important network of hydrogen bond interactions between the quinazolinone nucleus and the enzyme in which the C2-NH_2 moiety of CB3717 binds with the carbonyl oxygen of Ala263. A molecular surface analysis of this region of the active site shows that this interaction reduces access to a potentially large pocket accessible from the C2 position of the quinazolinone. In the C2-methyl series, where the hydrogen bond interaction with Ala263 does not exist, molecular modeling studies have shown that minor rearrangements of the last three C-terminal residues (Val262, Ala263, and Ile264)[38] allow access to this pocket without compromising the key binding interactions[37] between the C-terminal carboxylate of Ile264, Arg21 and a conserved molecule of water with N1 of the quinazoline (Figure 1).

The initial biological results on the 2-hydroxymethyl derivative were combined with the opportunities suggested by this analysis of the X-ray data and prompted us to investigate substitution at the C2 position of the quinazolinone nucleus more broadly. Compounds **4a**−**c** were used as comparator structures. The C2-methyl group (Scheme 1) was therefore substituted with O, N, S, Cl, and CN functionalities selected for their potential to access and bind in the C2 pocket and to improve the overall solubility while retaining the *m*-nitrobenzylamide substituent (Table 1).

In the 7,2′-unsubstituted[26] series (Table 1) most of the structural modifications led to molecules which inhibited the TS enzyme at submicromolar levels (IC_{50} 0.18−4.2 μM) (Table 1), but when compared to CB3717 (**1**) (IC_{50} 0.02 μM)[6] were 10−100-fold less potent. This reduced activity is partially explained by the loss of two key interactions in the enzyme/inhibitor complex: (1) the hydrogen bond interaction between the 2-amino group of **1** and the C-terminal residues[6,34] and (2) the

Table 2. *In Vitro* Activities of Acid-Containing Antifolates



compd	R^1	R^2	R^3	R^5 [a]	ref	IC_{50} (μM) inhibn of TS	IC_{50} (μM) inhibn of L1210 cell growth in culture
1	NH_2	H	H	(CH_2)COOH	6	0.02	5.0
2	H	H	H	$(CH_2)_2$COOH	6	0.05	0.15
41	OH	H	H	$(CH_2)_2$COOH	6	0.1	5
42	S-2-pyrimidine	H	H	$(CH_2)_2$COOH	6	0.24	100
43[b]	$N(CH_2CH_2)_2NCH_3$	CH_3	F	$(CH_2)_2$COOH[c]	39	0.028	>20
44	H	H	H	*m*-NO_2-benzyl	21	0.01	9

[a] *S*-isomer except otherwise stated. [b] See ref 43. [c] *R,S*.

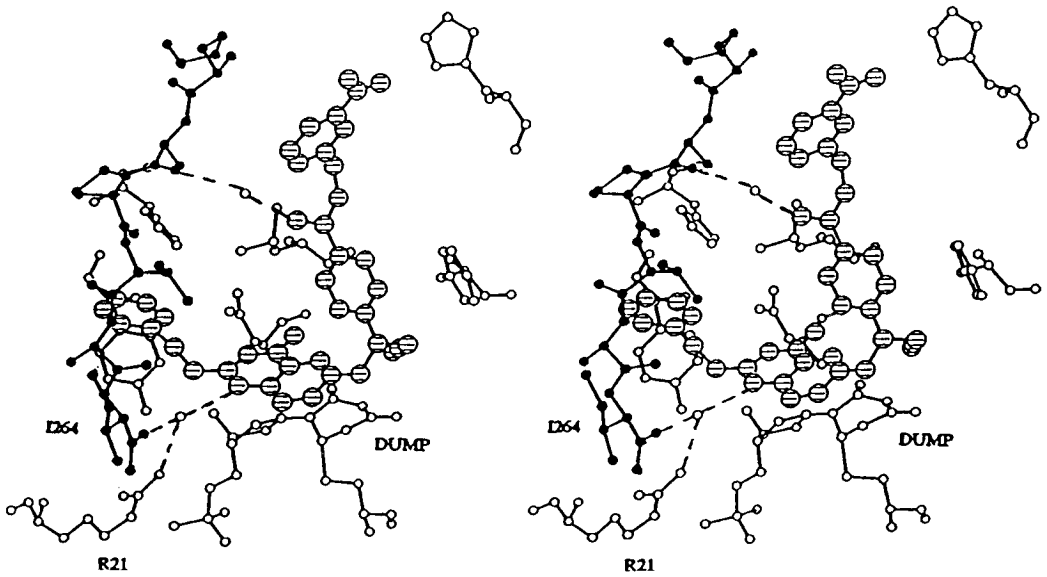


Figure 1. Stereodrawing of compound **35a** modeled in the active site of *E. coli*−TS ternary complex. **35a** is depicted with stippled circles; key hydrogen bonds are depicted with dashes lines. The C-terminal last residues are highlighted in full circles.

hydrogen bond interaction between the α-carboxylic acid of the glutamate residue and the enzyme.[36] The consequences of this latter interaction are illustrated by comparison of compounds **4a** and **44** (Tables 1 and 2), where **44** is a 10-fold better enzyme inhibitor than **4a**.

In nitrogen-containing C2 substituents, the tertiary amine **27a** is a 7- to >3-fold more potent TS inhibitor than the primary or secondary amino derivatives **28a** and **29a**. This result can be explained by the increased solvation of the amine function of the latter two compounds. Interestingly, analogues with large substituents such as *N*-methylpiperazine, morpholine, and hydroxyproline (**31**−**33a**) retained good enzyme inhibition and were equivalent to the smaller dimethylamino derivative **27a**, indicating that the enzyme can indeed accommodate fairly bulky substituents in the C2 region of the quinazolinone. This conclusion was further substantiated by the mercaptopyrimidine derivatives **35a**−**b**. The level of enzyme inhibition of these compounds (IC_{50} 0.48 and 0.11 μM) is only 2−4-fold lower than the equivalent C2-methyl analoges (**4a,b**, Table 1). Attempts to gain further interactions with the enzyme by substituting the pyrimidine nucleus with hydrogen bond donor substituents (NH_2, OH) failed and led to ∼2−7-fold reduction in the level of enzyme inhibition (**35a**, **36a**, **37a**, **38a**, Table 1). More flexible C2 substituents, as in the ((dimethylamino)ethyl)methylamino (**30b**) or bis(2-hydroxyethyl)amino (**34b**) derivatives were also well tolerated by the enzyme and gave compounds with IC_{50} values of 0.2 and 0.16 μM, respectively. Figure 1 shows the docking of the C2-CH_2-S-pyrimidine derivative **35a** into the ternary complex and illustrates that the pyrimidine nucleus can easily reach the C2 pocket without disturbing the fundamental network of hydrogen bond interactions.

As reported by our group, modeling studies[39,40] indicated that a 7-methyl substituent could reinforce a partially folded conformation of the *p*-aminobenzoate (PABA) ring inclined at an angle of 65° to the quinazolinone, believed to be optimum for binding to the TS enzyme. Moreover addition of a 2′-fluoro substituent to such systems gives a further 2−3-fold enhancement[7,9,25] in TS inhibition and growth inhibition, presumably through stabilization of the almost planar conformation in this region of the molecule due to a hydrogen bond between the fluorine atom and the amide N-H of the glutamate. When applied to the C2-modified series, this double substitution also proved to be beneficial. Comparison of **24b**, **27b**, **30b**, **31b**, **32b**, and **35b** with **24a**, **27a**, **30a**, **31a**, **32a**, and **35a**, respectively, clearly indicates that the fully substituted analogues are consistently 4−8-fold better enzyme inhibitors (IC_{50}

Table 3. Physicochemical Properties of C2-Modified Antifolates

compd	ClogP[a]	relative solubility[b,c]	compd	ClogP[a]	relative solubility[b,c]
4a	3.7[d]	1[e]	**32a**	3.06	5
24a	2.17	2	**35a**	4.03	10
27a	NC[f]	10	**37a**	4.60	0.5
29a	2.22	3	**38a**	NC[f]	0.5
31a	3.68	54			

[a] ClogP have been calculated by substracting from the measured value of **4a** the calculated contribution of 2-methyl-3,4-dihydro-6-methyloxoquinazoline (calculated using ClogP version 3.64, Daylight CIS, Irvine CA) and adding the calculated contributions (using ClogP version 3.64) of the C2-substituted-3,4-dihydro-6-methyl-4-oxoquinazoline. [b] For each compound, relative solubility is calculated as the ratio of its solubility over the solubility of **4a**. [c] Equilibrium solubility[44] measured at 3 days in 0.01 M aqueous NaH_2PO_4/0.15 M NaCl buffer at pH 7.4 and 25 °C. [d] Measured experimentally. [e] **4a**: equilibrium solubility = 0.12 μg/mL. [f] NC = not calculated.

0.072−0.2 μM). The improvement in potency seen with these changes is independent of the nature of the N10 substituent (propargyl or methyl) since **24c** and **27c** are almost equipotent to **24b** and **27b** (Table 1).

In terms of their ability to inhibit *in vitro* cell growth of the murine L1210 cell line as illustrated by **24a**, **27a**, **32a**, and **35a** (Table 1), the 7,2′-unsubstituted derivatives were equivalent to CB3717 (**1**) (IC_{50} 3.5 μM). This is, despite their being 10−100-fold less potent inhibitors of the isolated TS enzyme. The 4−8-fold improvements in enzyme inhibition observed in the C7-Me 2′-F derivatives **24b−c**, **27b−c**, **30b**, **31b**, **32b**, and **35b** also translates into improved *in vitro* potency against L1210 and leads to compounds which are ∼5−10-fold better inhibitors of cell growth than **1**. This improved expression of the enzyme inhibition into cell growth inhibition is explained by the fact that these non-acid-containing C2-modified TS inhibitors have lipophilicities in the range where diffusion through the cell membrane should be the preferred entry process (Table 3).

The high potency of these new C2-modified TS inhibitors clearly distinguishes them from their glutamic acid analogues in which, with the exception of the mono- and the difluoro derivatives,[6−8] the C2 modifications generally led to a dramatic loss in potency against the L1210 cell line. This conclusion is best reinforced by comparison of **31b** and **43** (Tables 1 and 2), where the non-acid-containing molecule **31b** shows a >35-fold better growth inhibition of L1210 *in vitro* despite being a 4-fold less potent TS inhibitor than **43**.

The lack of dependence of these series of compounds on the RFC to enter cells is strongly suggested by the consistently low L1210/L1210:1565 IC_{50} relative resistance ratios, measured on representative examples in the 7,2′-unsubstituted and 7-methyl-2′-fluoro series (Table 1).

Thymidine protection studies on representative examples confirmed that inhibition of TS is the predominant locus of cell growth inhibition. There are, however, two exceptions to this: **25a** and **31a** where thymidine protects against inhibition of cell growth only at twice but not at 10 times the IC_{50} values. This inability to completely protect the cells from high concentrations of compound suggests that inhibition of an alternative locus of action contributes to inhibition of cell growth (Table 1).

As shown in Table 3, introduction of substituents into the C2-methyl group resulted in an increase in solubility of up to 54-fold (measured at 3 days in phosphate buffer at 25 °C (pH 7.4)) when compared to the C2-methyl derivative **4a**. Even with bulky substituents, *e.g.* the mercaptopyrimidine nucleus **35a**, a 10-fold increase in solubility was achieved despite a slight increase in lipophilicity. However further functionalization of the pyrimidine ring by the introduction of hydrogen bond donor and acceptor moieties reduced the solubility by 10-fold (comparison of **35a** with **37a−38a**, Table 3). This is probably due to the combined effect of an increase in the overall log *P* resulting from proximity effects between the heteroatoms of the C2 substituent and the quinazolinone nucleus, and an increase in the melting point. C2 substitution with smaller hydrophilic substituents that easily form hydrogen bonds (*e.g.* OH in **24a** and NH_2 in **29a**, Table 3) only marginally (2−3-fold) increased the solubility compared to **4a** despite reducing the log *P* by ∼1.6 units. Substituents capable of being protonated at physiological pH (*e.g.* monoamines **27a** and **32a**) gave a 5−10-fold increase in solubility. This improvement is smaller than expected and is probably explained by the small proportion of the protonated form present at pH 7 due to the reduced basicity[41] of the C2 amine nitrogen atom resulting from the strong electron-withdrawing effect of the quinazolinone nucleus. The introduction of more basic amines (*e.g. N*-methylpiperazine **31a**[42]), more fully protonated at physiological pH, resulted in an increase in solubility of 54-fold compared to their C2-methyl counterpart (Table 3).

Conclusion

A series of novel antifolate analogues of **4a** in which the C2-methyl group has been substituted with N, O, S, Cl, and CN has been synthesized. These modifications result in compounds which are potent inhibitors of the isolated TS enzyme, which do not require the RFC for cell entry and which cannot be substrates for the FPGS enzyme. The most interesting examples in this series are active against the murine L1210 cell line with IC_{50} values ranging from 0.3 to 1 μM. The advantage in terms of this series when compared to the glutamic acid counterparts is clearly demonstrated by the >50-fold improved potency in cell growth inhibition of **31b** and **35a** compared to **43** and **42**, respectively.

The low aqueous solubility observed with lipophilic quinazoline antifolates, which results from the removal of carboxylic acid functions, has been overcome by the incorporation of suitable C2 substituents. This is best illustrated by the incorporation of an *N*-methylpiperazine nucleus into the C2-methyl group of **4a** leading to **31a** where a 54-fold increase in solubility has been achieved.

Experimental Section

General Procedures. All experiments were carried out under an inert atmosphere and at room temperature unless otherwise stated. *N*,*N*-Dimethylformamide (DMF) was purified by azeotropic distillation at 10 mmHg. Flash chromatography was carried out on Merck Kieselgel 60 (Art. 9385). The purities of compounds for test were assessed by analytical HPLC on a Hichrom S5ODS1 Sperisorb column system set to run isocratically with 60−70% MeOH + 0.2% CF_3COOH in H_2O as eluent. TLCs were performed on precoated silica gel plates (Merck Art. 5715), and the resulting chromatograms were visualized under UV light at 254 nm. Melting points were determined on a Kofler Block or with a Büchi melting

point apparatus and are uncorrected. The ^{1}H NMR spectra were determined in Me_2SO-d_6 solution (unless otherwise stated) on a Bruker AM 200 (200 MHz) spectrometer or on a JEOL JNM EX 400 (400 MHz). Chemical shifts are expressed in unit of δ (ppm), and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; br s, broad singlet; m, multiplet. Fast atom bombardment (FAB) mass spectra were determined with a VG MS9 spectrometer and Finnigan Incos data system, using Me_2SO as the solvent and glycerol as the matrix or with a Finnigan SSQ 7000 for the electrospray technique. With the appropriate mode either positive or negative ion data could be collected. NMR and mass spectra were run on isolated intermediates and final products and are consistent with the proposed structures. **11**, **13**, and **14** were prepared as described in ref 7.

2-(Chloromethyl)-3,4-dihydro-4-oxo-6,7-dimethylquinazoline (7). 2-Amino-4,5-dimethylbenzoic acid hydrochloride (4.5 g, 22.3 mmol) was added to a solution of sodium methoxide, prepared from Na (570 mg) in MeOH (50 mL). This solution was then poured into a solution of methyl chloroacetimidate prepared from chloroacetonitrile (1.88 g, 24.9 mmol) and sodium methoxide (prepared from Na (100 mg) in MeOH (50 mL). After stirring for 30 min the reaction mixture was heated at 80 °C for 2 h. After cooling to 0 °C, the resulting solid was filtered, washed with MeOH and then water, and dried under vacuum. The product was isolated as a white solid: 3.9 g (80%); NMR (Me_2SO-d_6/CD_3COOD) δ 2.35 (s, 3 H, CH_3), 2.37 (s, 3 H, CH_3), 4.53 (s, 2 H, CH_2), 7.47 (s, 1 H, quinazoline 8-H), 7.87 (s, 1 H, quinazoline 5-H); MS (CI) *m/z* 223, 225 $[MH]^+$. Anal. ($C_{11}H_{11}ClN_2O$) C, H, N.

The same procedure was repeated with 2-amino-5-methylbenzoic acid to give **5** in 85% yield.

2-(Acetoxymethyl)-3,4-dihydro-4-oxo-6,7-dimethylquinazolinone (8). A solution of **7** (22.5 g, 0.10 mol) in DMF (150 mL) containing sodium acetate (30 g, 0.36 mol) was heated at 80 °C for 3 h. After evaporation of the solvent the resulting solid was filtered, washed with petroleum ether and then water, and dried under vacuum: 23 g (93%); mp 210−212 °C dec; NMR (Me_2SO-d_6/CD_3COOD) δ 2.13 (s, 3 H, CH_3-CO), 2.34 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3), 4.93 (s, 2 H, CH_2O), 7.43 (s, 1 H, quinazoline 8-H), 7.86 (s, 1 H, quinazoline 5-H); MS (CI) *m/z* 247 $[MH]^+$. Anal. ($C_{13}H_{14}N_2O_3 \cdot 0.8H_2O$) C, H, N.

The same procedure was repeated with **5** to give **6** in 89% yield.

2-(Acetoxymethyl)-6-(bromomethyl)-3,4-dihydro-4-oxo-7-methylquinazolinone (10). A mixture of **8** (2.46 g, 10 mmol), NBS (2.13 g, 12 mmol), and benzoyl peroxide (100 mg) in CCl_4 (75 mL) containing cyclohexene oxide (100 mg, 1 mmol) was stirred under reflux and light for 2 h. On cooling an off-white precipitate was obtained which was filtered off. The organic layer was washed with water and brine, dried, and evaporated to dryness. The residue was purified by flash chromatography using a gradient of 30−50% v/v EtOAc in CH_2Cl_2 as eluent. The product was isolated as a white solid: 3.8 g (58%); NMR ($CDCl_3$) δ 2.27 (s, 3 H, CH_3CO), 2.57 (s, 3 H, CH_3), 4.61 (s, 2 H, CH_2Br), 5.14 (s, 2 H, CH_2O), 7.53 (s, 1 H, quinazoline 8-H), 8.19 (s, 1 H, quinazoline 5-H); MS (CI) *m/z* 325, 327 $[MH]^+$.

The same procedure was repeated with **6** to give **9** in 64% yield.

***tert*-Butyl 2-Fluoro-4-(trifluoroacetamido)benzoate (15).** To a solution of **12**[7] (10 g, 47 mmol) in CH_2Cl_2 (250 mL) containing Na_2CO_3 (15 g, 0.142 mol) was added trifluoroacetic anhydride (19.9 g, 95 mmol) over 10 min. After stirring for 2 h, the solid was filtered off. The filtrate was washed with water and brine and dried, and the solvent was evaporated. The crude oil was purified by flash chromatography using a gradient of 15−30% v/v Et_2O in hexane as eluent to give **15**: 13 g (89%); NMR δ 1.53 (s, 9 H, Bu^t), 7.6 (dd, 1 H, 3-H), 7.69 (dd, 1 H, 5-H), 7.86 (t, 1 H, 6-H), 11.63 (s, 1 H, NH); MS (CI) *m/z* 325 $[MH]^+$. Anal. ($C_{13}H_{13}F_4NO_3$) C, H, N.

***tert*-Butyl 2-Fluoro-4-(methylamino)benzoate (16).** To a solution of **15** (13 g, 42 mmol) in DMF (200 mL) was added Na_2CO_3 (70 g) followed by MeI (86 mL). After stirring overnight the solvent was evaporated. The crude residue was diluted with EtOAc and filtered. After evaporation of the filtrate to dryness, the resulting oil was dissolved in MeOH (100 mL) and 7% K_2CO_3 solution (50 mL) was added. Stirring was maintained for 3 h, and the mixture was evaporated to dryness. The resulting solid was partitioned between EtOAc and H_2O. The EtOAc solution was washed with brine, dried, and evaporated. After purification by flash chromatography using a gradient 30−50% Et_2O in hexane as eluent, **16** was isolated: 7.4 g (78%); mp 76−78 °C; NMR (Me_2SO-d_6/CD_3-COOD) δ 1.49 (s, 9 H, Bu^t), 2.71 (s, 3 H, CH_3), 6.25 (dd, 1 H, 3-H), 6.38 (dd, 1 H, 5-H), 7.55 (dd, 1 H, 6-H); MS (CI) *m/z* 226 $[MH]^+$. Anal. ($C_{12}H_{16}FNO_2$) C, H, N.

2-Fluoro-4-(methylamino)benzoic Acid (19). A solution of **16** (4 g, 17.8 mmol) in 1/1 CH_2Cl_2/TFA (60 mL) was stirred for 3 h. After evaporation of the solvent, the oil was triturated in ether and the resulting solid was filtered off: 2.5 g (84%); mp 184−186 °C; NMR (Me_2SO-d_6/CD_3COOD) δ 2.72 (s, 3 H, CH_3), 6.28 (dd, 1 H, 3-H), 6.40 (dd, 1 H, 5-H), 7.65 (dd, 1 H, 6-H); MS (CI) *m/z* 187 $[M + NH_4]^+$. Anal. ($C_8H_8FNO_2 \cdot 0.16CF_3$-COOH) C, H, N.

The same procedure was repeated with **13** and **14** to give **17** and **18**, respectively.

1-[[*N*-[2-Fluoro-4-(prop-2-ynylamino)benzoyl]amino]-methyl]-3-nitrobenzene (21). To a solution of **18** (2.4 g, 12 mmol) and *m*-nitrobenzylamine (4.6 g, 25 mmol) in DMF (40 mL) at 0 °C was added dropwise DPPA (2.58 mL) followed by Et_3N (11.7 mL). After stirring for 16 h the solvent was evaporated. The crude oil was dissolved in EtOAc. The organic layers were washed with water and brine, dried, and evaporated. After purification by flash chromatography using a gradient 30−50% v/v EtOAc in CH_2Cl_2 as eluent, **21** was isolated as a solid: 3.1 g (79%); mp 168−170 °C; NMR (Me_2-SO-d_6/CD_3COOD) δ 3.10 (s, 1 H, C≡CH), 3.95 (s, 2 H, $CH_2C{\equiv}C$), 4.56 (d, 2 H, $NHCH_2$), 6.45 (dd, 1 H, 3′-H), 6.52 (dd, 1 H, 5′-H), 7.55 (dd, 1 H, 6′-H), 7.62 (dd, 1 H, NO_2-benzyl 5-H), 7.80 (d, 1 H, NO_2-benzyl 6-H), 8.15 (dd, 1 H, NO_2-benzyl 4-H), 8.2 (s, 1 H, NO_2-benzyl 2-H), 8.5 (dd, 1 H, HNCO); MS (FAB) *m/z* 328 $[MH]^+$. Anal. ($C_{17}H_{14}FN_3O_3$) C, H, N.

The same procedure was applied to **17** and **19** to give **20** and **22**, respectively.

1-[[*N*-[4-[*N*-[[2-(Acetoxymethyl)-3,4-dihydro-7-methyl-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]methyl]-3-nitrobenzene (23b). A mixture of **21** (1.68 g, 5.3 mmol), the bromomethyl compound **10** (1.73 g, 5.3 mmol), and $CaCO_3$ (445 mg, 7.95 mmol) in DMF (50 mL) was stirred at 80 °C for 18 h. After evaporation of the solvent, the solid was triturated with water and filtered. The crude product was purified by flash chromatography using a gradient 1:1 to 1:9 v/v CH_2Cl_2/EtOAc as eluent to give **23b**: 1.78 g (60%); NMR δ 2.11 (s, 3 H, CH_3CO), 2.44 (s, 3 H, CH_3), 3.2 (s, 1 H, C≡CH), 4.30 (s, 2 H, $CH_2C{\equiv}CH$), 4.55 (d, 2 H, $NHCH_2$), 4.7 (s, 2 H, CH_2N), 4.91 (s, 2 H, OCH_2), 6.6 (dd, 1 H, 3′-H), 6.7 (d, 1 H, 5′-H), 7.5 (s, 1 H, quinazoline 8-H), 7.58 (t, 1 H, 6′-H), 7.62 (t, 1 H, NO_2-benzyl 5-H), 7.72 (s, 1 H, quinazoline 5-H), 7.75 (d, 1 H, NO_2-benzyl 6-H), 8.1 (dd, 1 H, NO_2-benzyl 4-H), 8.15 (s, 1 H, NO_2-benzyl 2-H), 8.55 (dd, 1 H, NH); MS (FAB) *m/z* 572 $[MH]^+$.

The same procedure was applied to **20** and **22** to give **23a** (78%) and **23c** (44%), respectively.

1-[[*N*-[4-[[3,4-Dihydro-2-(hydroxymethyl)-7-methyl-4-oxo-6-quinazolinyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-amino]methyl]-3-nitrobenzene (24b). A solution of **23b** (50 mg, 0.08 mmol) in 1 N NaOH (5 mL) and MeOH (1 mL) was stirred for 4 h. After acidification to pH 5 with 2 N HCl, the suspension was centrifuged. The solid pellet was washed thoroughly with H_2O and freeze dried to give **24b** as a white solid: 40 mg (89%); NMR (Me_2SO-d_6/CF_3COOD) δ 2.55 (s, 3 H, CH_3), 3.28 (t, 1 H, C≡CH), 4.4 (s, 2 H, CH_2 C≡C), 4.56 (s, 2 H, $NHCH_2$), 4.70 (s, 2 H, CH_2O), 4.80 (s, 2 H, CH_2N), 6.65 (d, 1 H, 3′-H), 6.7 (d, 1 H, 5′-H), 7.62 (dd, 1 H, 6′-H), 7.65 (dd, 1 H, NO_2-benzyl 5-H), 7.80 (d, 1 H, NO_2-benzyl 6-H), 7.82 (s, 1 H, quinazoline 8-H), 7.88 (s, 1 H, quinazoline 5-H), 8.12 (dd, 1 H, NO_2-benzyl 4-H), 8.2 (s, 1 H, NO_2-benzyl 2-H), 8.62 (dd, 1 H, CONH); MS (FAB) *m/z* 530 $[MH]^+$. Anal. ($C_{28}H_{24}FN_5O_5 \cdot$ 2NaCl) C, H, N.

The same procedure was repeated with **23a,c** to give **24a** (74%) and **24c** (70%), respectively.

1-[[*N*-[4-[*N*-[[2-(Chloromethyl)-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]amino]methyl]-3-nitrobenzene (25a). A solution of **24a** (200 mg, 0.40 mmol) in CH_2Cl_2 (5 mL) containing $SOCl_2$ (86 μL) was stirred for 1 h. After evaporation to dryness the solid was triturated with ether and filtered off. The solid was purified by flash chromatography using a gradient of 1:1 to 1:9 v/v CH_2Cl_2/EtOAc as eluent to give **25a**: 180 mg (86%); NMR δ 3.25 (t, 1 H, C≡CH), 4.35 (s, 2 H, CH_2C≡C), 4.52 (s, 2 H, CH_2Cl), 4.55 (d, 2 H, $NHCH_2$), 4.8 (s, 2 H, CH_2N), 6.85 (d, 2 H, 3′-H and 5′-H), 7.65 (dd, 1 H, NO_2-benzyl 5-H), 7.67 (d, 1 H, quinazoline 8-H), 7.80 (d, 4 H, 2′-H, 6′-H, quinazoline 7-H and NO_2-benzyl 6-H), 8.0 (s, 1 H, quinazoline 5-H), 8.15 (d, 1 H, NO_2-benzyl 4-H), 8.17 (s, 1 H, NO_2-benzyl 2-H), 8.85 (t, 1 H, NH); MS (FAB) *m/z* 516 $[MH]^+$. Anal. ($C_{27}H_{22}ClN_5O_4 \cdot 1.1H_2O$) C, H, N.

The same procedure was applied to **24b,c** to give **25b,c**, respectively, which were used in the next step without purification. In the case of **25b** and **25c** the NMR and mass spectra are consistent with those of the assigned structures.

1-[[*N*-[4-[*N*-[[3,4-Dihydro-2-[(dimethylamino)methyl]-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]amino]methyl]-3-nitrobenzene (27a). Method A. To a saturated solution of dimethylamine in MeOH (15 mL) was added **25a** (150 mg, 0.29 mmol). After 2 h the solvent was evaporated. The residue was triturated with ether, and the resulting solid was filtered off and washed with water. Purification was achieved by preparative HPLC using a gradient 20:80 to 40:60 of CH_3CN/H_2O (0.1% CF_3COOH) v/v. The appropriate fractions were combined and the CH_3CN was removed by rotary evaporation. Lyophilization of the residual solution gave **27a** as a white solid: 71 mg (47%); NMR δ 2.98 (s, 6 H, NMe_2), 3.15 (t, 1 H, C≡CH), 4.3 (s, 4 H, CH_2NMe_2 and CH_2C≡C), 4.55 (d, 2 H, $NHCH_2$), 4.8 (s, 2 H, CH_2N), 6.85 (d, 2 H, 3′-H and 5′-H), 7.62 (t, 1 H, NO_2-benzyl 5-H), 7.65 (d, 1 H, quinazoline 8-H), 7.75 (d, 4 H, 2′-H, 6′-H, quinazoline 7-H and NO_2-benzyl 6-H), 8.05 (d, 1 H, quinazoline 5-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.15 (s, 1 H, NO_2-benzyl 2-H), 8.85 (t, 1 H, NH), 12.5 (s, 1 H, NH); MS (FAB) *m/z* 525 $[MH]^+$. Anal. ($C_{29}H_{28}N_6O_4 \cdot 1.65CF_3COOH$) C, H, N.

The same procedure was repeated with the appropriate amines to give **28a** (39%), **29a** (27%), **30a** (32%), **31a** (65%), **32a** (53%), and **33a** (31%).

1-[[*N*-[4-[*N*-[[3,4-Dihydro-2-[(dimethylamino)methyl]-7-methyl-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]methyl]-3-nitrobenzene (27b). Method B. To a solution of **25b** (210 mg, 0.38 mmol) in MeOH (2 mL) was added a saturated solution of dimethylamine in MeOH (5 mL). After stirring at 80 °C for 30 min the solvent was evaporated, and the residue was purified by flash chromatography on silica using 3:97 v/v MeOH/$CHCl_3$ as eluent to yield **27b**: 120 mg (56%); NMR (Me_2SO-d_6/CF_3COOD) δ 2.55 (s, 3 H, CH_3), 2.97 (s, 6 H, $(CH_3)_2N$), 3.25 (s, 1 H, C≡CH), 4.38 (s, 4 H, N-CH_2C≡C and Me_2NCH_2), 4.6 (s, 2 H, $NHCH_2$), 4.8 (s, 2 H, CH_2N), 6.65 (d, 1 H, 3′-H), 6.68 (d, 1 H, 5′-H), 7.60 (s, 1 H, quinazoline 8-H), 7.62 (dd, 1 H, 6′-H), 7.65 (dd, 1 H, NO_2-benzyl 5-H), 7.78 (s, 1 H, quinazoline 5-H), 7.85 (d, 1 H, NO_2-benzyl 6-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.2 (s, 1 H, NO_2-benzyl 2-H); MS (ESI) 557 $[MH]^+$. Anal. ($C_{30}H_{29}FN_6O_4 \cdot H_2O$) C, H, N.

The same procedure was repeated with the appropriate amines to give **30b** (35%), **31b** (31%), **32b** (63%), and **34b** (54%).

1-[[*N*-[4-[*N*-[[3,4-Dihydro-2-[(dimethylamino)methyl]-7-methyl-4-oxo-6-quinazolinyl]methyl]-*N*-methylamino]-2-fluorobenzoyl]amino]methyl]-3-nitrobenzene (27c). Method C. To a solution of **25c** (210 mg, 0.4 mmol) in MeOH (3 mL) was added 33% Me_2NH in MeOH (7 mL). After stirring 2.5 h at 80 °C the solution was evaporated to dryness. The residue was purified by flash chromatography using a gradient MeOH/$CHCl_3$ 1:99 to 3:97 v/v as eluent: 89 mg (42%); NMR (Me_2SO-d_6/CF_3COOD) δ 2.5 (s, 3 H, CH_3), 2.97 (s, 6 H, Me_2N), 3.15 (s, 3 H, CH_3N), 4.35 (s, 2 H, Me_2NCH_2), 4.58 (s, 2 H, $NHCH_2$), 4.78 (s, 2 H, CH_2N), 6.55 (d, 1 H, 3′-H), 6.6 (d, 1 H, 5′-H), 7.6 (s, 2 H, quinazoline 5-H and 8-H), 7.62 (dd, 1 H, 6′-H), 7.65 (dd, 1 H, NO_2-benzyl 5-H), 7.8 (d, 1 H, NO_2-benzyl 6-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.2 (s, 1 H, NO_2-benzyl 2-H); MS (FAB) *m/z* 533 $[MH]^+$. Anal. ($C_{28}H_{29}FN_6O_4 \cdot 0.8H_2O$) C, H, N.

1-[[*N*-[4-[*N*-[[3,4-Dihydro-7-methyl-4-oxo-2-[(2-pyrimidinylthio)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]methyl]-3-nitrobenzene (35b). Method E. 2-Mercaptopyrimidine (61 mg, 0.54 mmol) was added to 60% NaH (27 mg) in DMF (15 mL). After stirring for 30 min **25b** (290 mg, 0.54 mmol) was added, and stirring was continued for 24 h. After evaporation of the solvent the residue was partitioned between EtOAc/water. The organic layers were washed with water and brine, dried, and evaporated. The residue was purified by flash chromatography on silica using a gradient 25:75 to 60:40 v/v EtOAc/CH_2Cl_2 as eluent: 60 mg (84%); NMR δ 2.43 (s, 3 H, CH_3), 3.2 (s, 1 H, C≡CH), 4.29 (s, 2 H, CH_2C≡C), 4.38 (s, 2 H, SCH_2), 4.57 (d, 2 H, $NHCH_2$), 4.69 (s, 2 H, CH_2N), 6.62 (d, 1 H, 5′-H), 6.7 (s, 1 H, 3′-H), 7.22 (t, 1 H, pyrimidine 5-H), 7.47 (s, 1 H, quinazoline 8-H), 7.5−7.65 (m, 2 H, 6′-H and NO_2-benzyl 5-H), 7.72 (s, 1 H, quinazoline 5-H), 7.8 (d, 1 H, NO_2-benzyl 6-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.2 (s, 1 H, NO_2-benzyl 2-H), 8.5−8.6 (m, 1 H, NH), 8.65 (d, 2 H, pyrimidine 4-H and 6-H); MS (FAB) *m/z* 624 $[MH]^+$. Anal. ($C_{32}H_{26}FN_7O_4S \cdot 1.75H_2O$) C, N; H: calcd, 4.54; found, 4.1.

The same procedure was repeated with **25a** in place of **25b** to give **35a** (66%) (method D). Applied to the appropriate thiols, method D gave **36a** (63%), **37a** (56%), and **38a** (66%).

1-[[*N*-[4-[*N*-[[3,4-Dihydro-2-[(2-methoxyethoxy)methyl]-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]amino]methyl]-3-nitrobenzene (26a). Method F. To a suspension of 60% NaH (64 mg) in DMF (4 mL) was added 2-methoxyethanol (3 mL). After stirring for 30 min **25a** (206 mg, 0.4 mmol) in DMF (2 mL) was added, and stirring was continued for 18 h. After evaporation to dryness, the residue was triturated with water and the resulting solid was filtered off. Purification by flash chromatography using a gradient 2:98 to 4:96 v/v MeOH/$CHCl_3$ as eluent gave **26a**: 41 mg (18%); NMR δ 3.15 (t, 1 H, C≡CH), 3.25 (s, 3 H, OCH_3), 3.5 (dd, 2 H, OCH_2), 3.65 (dd, 2 H, OCH_2), 4.33 (d, 2 H, CH_2C≡C), 4.39 (s, 2 H, CH_2O), 4.55 (d, 2 H, $NHCH_2$), 4.8 (s, 2 H, $ArCH_2N$), 6.85 (d, 2 H, 3′-H and 5′-H), 7.6 (d, 2 H, quinazoline 8-H and NO_2-benzyl 5-H), 7.75 (dd, 1 H, quinazoline 7-H), 7.8 (d, 1 H, NO_2-benzyl 6-H), 7.85 (dd, 2 H, 2′-H and 6′-H), 8.0 (d, 1 H, quinazoline 5-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.2 (s, 1 H, NO_2-benzyl 2-H), 12.0 (s, 1 H, NH); MS (FAB) *m/z* 556 $[MH]^+$. Anal. ($C_{30}H_{29}N_5O_6 \cdot 0.66H_2O$) C, H, N.

1-[[*N*-[4-[*N*-[[2-(Cyanomethyl)-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]amino]methyl]-3-nitrobenzene (39a). Method G. To a solution of **25a** (155 mg, 0.3 mmol) in DMSO (8 mL) was added KCN (78 mg, 12 mmol). After stirring for 3 h the solvent was evaporated. The residue was taken into water, and the pH was adjusted to 5 with acetic acid. The resulting solid was filtered off and purified by flash chromatography using EtOAc as eluent to give **39a**: 33 mg (22%); NMR δ 3.15 (t, 1 H, C≡CH), 4.11 (s, 2 H, CH_2CN), 4.3 (s, 2 H, CH_2C≡C), 4.55 (d, 2 H, $NHCH_2$), 4.8 (s, 2 H, CH_2N), 6.85 (d, 2 H, 3′-H and 5′-H), 7.55−7.7 (m, 2 H, NO_2-benzyl 6-H and NO_2-benzyl 5-H), 7.75 (d, 4 H, 2′-H, 6′-H and quinazoline 7-H, 8-H), 8.0 (d, 1 H, quinazoline 5-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.15 (s, 1 H, NO_2-benzyl 2-H), 8.85 (t, 1 H, NHCO); MS (FAB) *m/z* 507 $[MH]^+$. Anal. ($C_{28}H_{22}N_6O_4 \cdot 0.9H_2O$) C, H, N.

1-[[*N*-[4-[*N*-[[3,4-Dihydro-2-[(*N*-methanesulfonamido)-methyl]-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]amino]methyl]-3-nitrobenzene (40a). Method H. Methanesulfonyl chloride (32 μL, 0.4 mmol) was added to a solution of **29a** (200 mg, 0.4 mmol) in CH_3CN (15 mL). After addition of Et_3N (56 μL, 0.4 mmol) the reaction mixture was stirred for 4 h. After filtration to remove the precipitate the filtrate was evaporated to dryness, the residue was triturated with H_2O, filtered off, and dried under vacuum. Purification by preparative HPLC using a gradient 30:70 to 70:30 CH_3CN/H_2O (0.1% CF_3COOH) as eluent gave **40a**: 40 mg (17%); NMR δ 3.0 (s, 3 H, CH_3SO_2), 3.15 (s, 1 H, C≡CH), 4.15 (s, 2 H, SO_2-

$NHCH_2$), 4.3 (s, 2 H, $CH_2C{\equiv}C$), 4.55 (d, 2 H, $NHCH_2$), 4.8 (s, 2 H, CH_2N), 6.85 (d, 2 H, 3′-H and 5′-H), 7.63 (d, 2 H, quinazoline 8-H and NO_2-benzyl 5-H), 7.75 (dd, 1 H, quinazoline 7-H), 7.76 (d, 2 H, 2′-H and 6′-H), 7.8 (d, 1 H, NO_2 benzyl 6-H), 8.0 (d, 1 H, quinazoline 5-H), 8.1 (d, 1 H, NO_2-benzyl 4-H), 8.15 (s, 1 H, NO_2-benzyl 2-H), 8.85 (t, 1 H, NH); MS (FAB) *m/z* 575 $[MH]^+$. Anal. ($C_{28}H_{26}N_6O_6S{\cdot}0.25CF_3COOH$) C, H, N.

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(41) 2-[(Dimethylamino)methyl]-3,4-dihydro-6-methyl-4-oxoquinazoline: pK_a (NMe_2) = 6.95.
(42) 2-[(*N*-Methylpiperazin-4-yl)methyl]-3,4-dihydro-6-methyl-4-oxoquinazoline: pK_a (MeN) = 9.8.
(43) **43** was prepared by coupling **10** and diethyl *N*-[2-fluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate[7] as described in ref 7. Subsequent functionalization of the C2 position was achieved as described for compound **31b**. **43**: NMR (Me_2SO-d_6/CD_3COOD) δ 1.9−2.0 (m, 1 H, CH_2), 2.0−2.2 (m, 1 H, CH_2), 2.25 (t, 2 H, 2 CH_2COOH), 2.5 (s, 3 H, CH_3), 2.65 (s, 3 H, NCH_3), 2.7−2.8 (s, 4 H, CH_2N), 3.0−3.1 (s, 4 H, CH_2N), 3.15 (s, 1 H, C≡CH), 3.55 (s, 2 H, CH_2N), 4.3 (s, 2 H, $CH_2C{\equiv}C$), 4.4 (dd, 1 H, CHCOOH), 4.75 (s, 2 H, NCH_2), 6.65 (dd, 1 H, 3′-H), 6.7 (dd, 1 H, 5′-H), 7.52 (s, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, 6′-H), 7.8 (s, 1 H, quinazoline 5-H); MS (FAB) *m*/*z* 605 $[MH]^-$. Anal. ($C_{31}H_{35}FN_6O_6 \cdot 1.3H_2O$) C, H, N.
(44) The solubilities have been measured by Dr. D. Leahy's group of Zeneca Pharmaceuticals.

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